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Mount Imlay – an island of floristic significance on the brink

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Abstract: Mount Imlay is an isolated summit in south-eastern New South Wales, rising to 886 m above sea level. In addition to its cultural significance and tourism value, the summit is home to three endemic plant species (*Boronia imlayensis, Eucalyptus imlayensis* and *Hibbertia circinata*) and several regionally rare or disjunct species. *Eucalyptus imlayensis* has been identified as one of 100 priority threatened species for conservation efforts in the coming years. Mount Imlay was burnt severely in the fire of 2019 / 20 and faces the threat of more frequent fire in the future, resulting from climate change. Many species are threatened by the introduced pathogen *Phytophthora cinnamomi*, which is now present on most of the mountain. In this paper, we outline the work done to date on protecting Mount Imlay's significant flora and identify plant species likely to be eligible for listing in threatened species legislation; currently, only two of the species are listed as threatened in NSW. Because most significant species on Mount Imlay share the same threats, the costs of future conservation work can be shared and undertaken regardless of legislative status. For instance, spraying with phosphite to reduce the threat from *Phytophthora cinnamomi* for one species will benefit many species.

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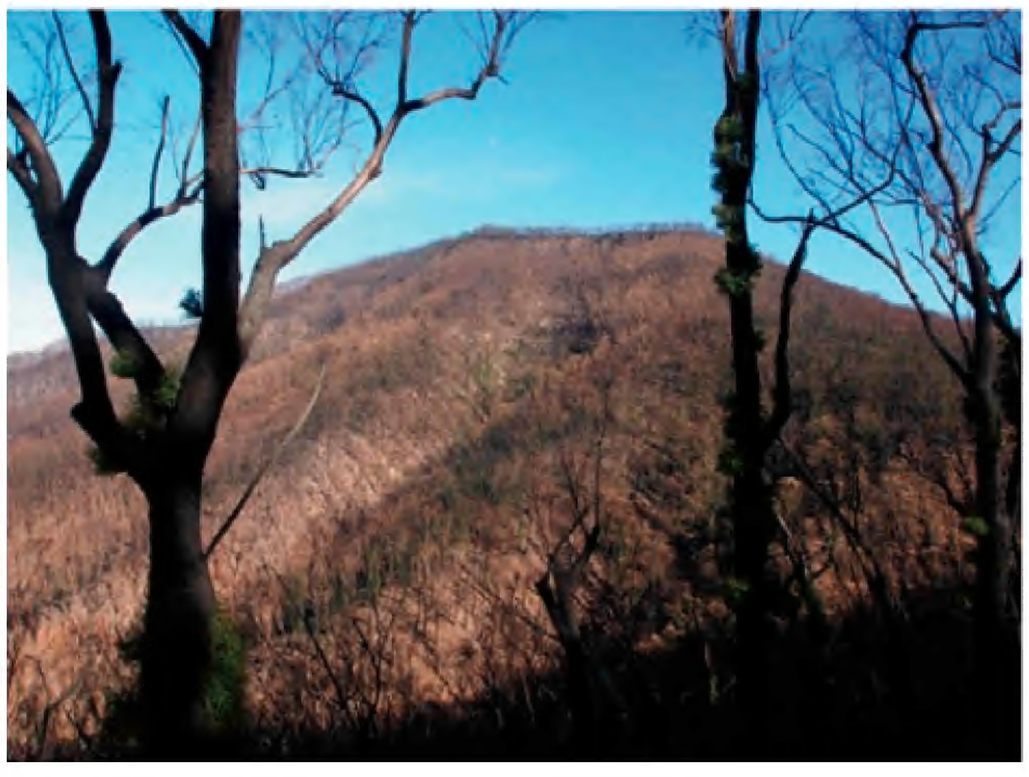
Introduction

Mount Imlay is an isolated peak rising to 886 m a.s.l. about 20 km south-west of Eden in south-eastern New South Wales (Figure 1). The slopes of the mountain are within Mount Imlay National Park and surrounded by state forest, which is subject to harvesting of native timber. The summit area forms a narrow rocky spine with cliffs of Devonian sedimentary rocks on all but the southern aspect. The peak is culturally significant (Department of Agriculture, Water and the Environment 2022a). Mount Imlay is considered an Asset of Intergenerational Significance for two endemic plants species, *Eucalyptus imlayensis* and *Hibbertia circinata*, under the amended NSW *National Parks and Wildlife Act 1974*.



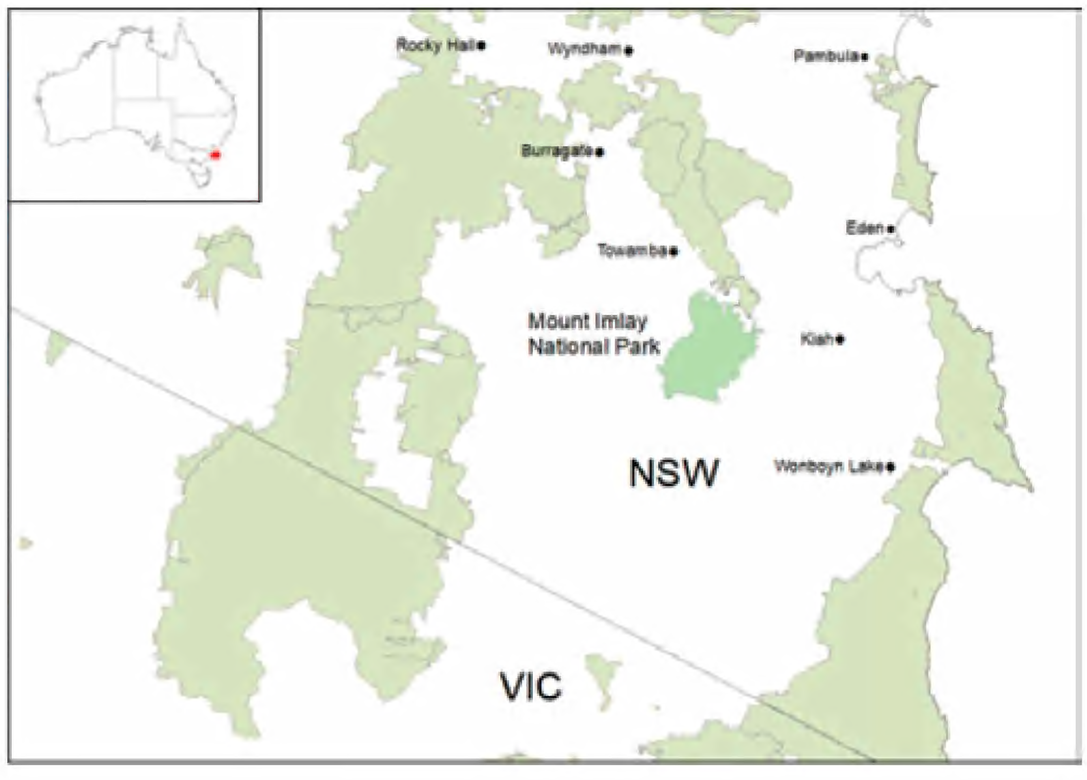


Figure 1. Mount Imlay is an isolated peak south-west of Eden, in Mount Imlay National Park, New South Wales. The narrow summit ridge is above 800 m a.sl.

The lower slopes of Mount Imlay National Park are dominated by *Eucalyptus sieberi* with a shrubby understorey. Gullies typically contain *Eucalyptus cypellocarpa* and species of wet sclerophyll forest. The summit area is fringed by a forest of *Eucalyptus fraxinoides* on the eastern and northern sides, while the rocky summit itself has scattered stunted *Eucalyptus sieberi*. The steep, eastern slope below the summit features a shrubland dominated by *Leptospermum scoparium* with emergent *Eucalyptus sieberi*, *Eucalyptus fraxinoides* and *Eucalyptus imlayensis*. This highly localised shrubland appears to be restricted to the mountain. The shrubland habitat is unusually wet for this dry mountain, receiving regular dew and mist from coastal winds in summer. There is very little soil in this community but mosses (including *Sphagnum australe*) are abundant on the rocks and in crevices.

A popular walking track leads to the summit from the north-eastern side of the mountain but there was once a rough vehicle track (now overgrown) to the summit from the south-western side. There is a small communications tower on the summit. The pathogen *Phytophthora cinnamomi* was isolated on the summit in 1999 prior to the construction of the tower. It has transformed understorey vegetation of the forest on the lower northern slopes from a diverse shrub-dominated community to a species-poor graminoid-dominated community (McDougall and Summerell 2003). *Xanthorrhoea australis* is the most obviously affected species, with these arborescent monocots turning yellow as they succumb. Until the early 2000s they were present close to the summit itself but have gradually died out there. *Phytophthora cinnamomi* has been recovered from the roots of the following species on Mt Imlay since 2000: *Amperea xiphoclada, Banksia spinulosa* var. *cunninghamii*, *Boronia imlayensis, Dillwynia glaberrima, Diplarrena moraea, Gonocarpus teucrioides, Hibbertia circinata, Leucopogon affinis, Monotoca glauca, Philotheca virgata, Platysace lanceolata, Prostanthera walteri, Stylidium armeria, Tetratheca subaphylla* and *Xanthorrhoea australis* (McDougall and Summerell 2003, Liew and McDougall unpublished data). Species such as *Epacris impressa* and *E. microphylla* are also likely to be affected; symptomatic plants of the former have not been sampled on Mount Imlay but the species is known to be highly susceptible elsewhere (Weste 2001), while, prior to the fire of 2019 /20, the latter only grew in areas where the pathogen was likely to be absent and had perhaps not yet come into contact with it. *Phytophthora cinnamomi* may also contribute to occasional deaths of *Eucalyptus sieberi* and *Eucalyptus obliqua* on the lower slopes. Both species are known to be susceptible but, given the dominance of at least *Eucalyptus sieberi* where *Phytophthora cinnamomi* is also abundant, susceptibility is probably low. The wet summer of 2021 / 22 resulted in more deaths in more species than usual. *Leucopogon affinis* for instance was thought to be largely unaffected by *Phytophthora cinnamomi* (McDougall 2006) but was commonly seen dead and dying in early 2022, and the pathogen was recovered from the roots of symptomatic plants. Apart from the northern part of the summit ridge and isolated pockets of healthy *Xanthorrhoea australis*, most of the park is likely to be infested with *Phytophthora cinnamomi*. Sadly, in the years since the recent fire, the pathogen appears to have made its way onto the summit ridge, where most of the significant plants grow.

Most of Mount Imlay National Park was burnt in a wildfire in late December 2019 / early January 2020, which moved rapidly through the park from the south. The eastern and southern aspects, typically moist and probably somewhat resistant to burning, had become dry during the drought in the preceding years and burnt severely (Figure 2). The fire was so hot there that it melted metal markers and consumed the lignotubers of young *Eucalyptus imlayensis* planted in 2011 and 2018. The fire, coupled with the increased activity of *Phytophthora cinnamomi* in the years since and its apparent spread to previously uninfested areas, has heightened the threat to the area's flora and vegetation. Currently, two plant species are listed as threatened under the Commonwealth *Environment Protection and Biodiversity Act 1999 (EPBC Act)* and the New South Wales *Biodiversity Conservation Act 2016 (BC Act): Eucalyptus imlayensis and Hibbertia circinata.* A few species are listed in Victoria under the *Flora and Fauna Guarantee Act 1988 (FFG Act)*, an indication of their broader conservation significance. We believe that other species warrant inclusion in NSW threatened species legislation. In this brief communication, we review the significant plant species of Mount Imlay National Park and describe the conservation work done to date by the NSW Department of Planning and Environment (DPE), Australian National Botanic Gardens (ANBG), Booderee Botanic Gardens (BBG) and the Royal Botanic Gardens Victoria, and that required in the future.

Figure 2. The steep eastern face of Mt Imlay on the 13th of January, 13 days after a fire, which burnt the entire *Eucalyptus imlayensis* population there. The fire burnt into the soil A horizon in places. Photo: Grant Brewer, NSW National Parks and Wildlife Service.

Species of significance

Boronia imlayensis

Status: EPBC Act - not listed; BC Act – not listed; FFG Act – not relevant

Population size: pre-fire 1000s, post-fire 10s of thousands

Susceptibility to Phytophthora cinnamomi: susceptible

Fire response: obligate seeder (killed by fire)

Boronia imlayensis is a shrub that grows to about 1.5 m tall (Figure 3a). It is endemic to Mount Imlay, where it is abundant but restricted to the northern part of the summit ridge and adjoining slopes above the cliffs (Figure 3b), mostly where *Phytophthora cinnamomi* has until recently been absent. It is an obligate seeder, its entire population having been burnt and killed in the fire of 2019 / 20. Seedlings were not apparent in June 2020 six months after the fire but were numerous by November 2020; the population now undoubtedly exceeds the pre-fire number and is continuing to recruit. About 5% of the population flowered in spring 2021 but seed production was not assessed after that event. The species is susceptible to *Phythophthora cinnamomi* and may be killed by the pathogen, but appears to be much less susceptible than *Hibbertia circinata* with which it grows. The long-term effect of *Phytophthora cinnamomi* on the *Boronia imlayensis* population will depend on whether its recruitment exceeds its mortality. The species should be assumed to be at risk from this pathogen unless population monitoring assesses it as being able to persist despite occasional deaths.

Figure 3. a) The pink flowers and pinnate foliage of *Boronia imlayensis* (Photo: Andrew Morrison); b) this species grows up to 1.5 m tall with another endemic *Hibbertia circinata* (yellow flowers) on the summit ridge of Mount Imlay.

Cuttings were taken in July 2018 by staff of the ANBG for the establishment of an ex-situ conservation population. There was very limited success with propagation, and trials over a 15-year period have shown this species to be extremely difficult to propagate vegetatively. In autumn 2021, a small number of seedlings growing in places on Mount Imlay where they were unlikely to survive (e.g. along the walking track and where very dense) were removed and distributed between the ANBG and BBG to add to the small existing ex-situ populations. Future propagation of this species for translocation or ex-situ conservation will certainly be dependent on seed availability. However, at present, no seed is held in conservation seed banks, and seed storage requirements and germination cues for the species are not known.

Based on area of occupancy, extent of occurrence, fluctuations in population and threats, this species is likely to be eligible for listing as critically endangered under the EPBC Act according to guidelines for assessing the conservation status of native species (Department of Agriculture, Water and the Environment undated).

Eucalyptus imlayensis

Status: EPBC Act – critically endangered; BC Act – critically endangered; FFG Act – not relevant

Population size: post fire, 48 individuals representing 17 genets

Susceptibility to Phytophthora cinnamomi: apparently not susceptible based on limited glasshouse tests and baiting of root material collected in the field

Fire response: resprouts from lignotuber after fire

Eucalyptus imlayensis is a multi-stemmed tree to 7 m tall, arising from a large lignotuber. It is endemic to Mount Imlay, where it is restricted to an area of approximately 3000 m^2 on the eastern face of the summit ridge. The species was identified in 2022 as one of 100 priority threatened species in Australia's Threatened Species Strategy Action Plan 2021 – 2026 (Department of Agriculture, Water and the Environment 2022b). Under this plan, in 2026, *Eucalyptus imlayensis* will be on track for improved trajectory by 2031.

At the first census of the population in 2001, there were thought to be about 80 individuals. Despite the small area in which they grow, an accurate count of the population was not possible until after the 2019 / 20 fire because of the difficult, steep terrain and dense growth of *Leptospermum scoparium*. James and McDougall (2007) found five genotypes in a sample of 27 accessible plants. The fire in 2019 / 20 allowed all plants to be easily accessed, tagged and sampled for further genetic analysis. In 2022, there were found to be 48 plants but only 17 genetic individuals (Simmons and James 2022). The clones range in size from 1 m^2 to about 235 m^2. One of the genets identified in 2007 (represented by four plants) has since died, but the cause of death is unknown.

Eucalyptus imlayensis is threatened primarily because it will be many years until resprouting plants are reproductive and there was very little seed in storage prior to the fire, and so, without seed storage, any catastrophic event that destroys regenerating plants may lead to extinction. Before the fire, plants rarely produced seed although they often flowered. Plants resprouted well after the fire, with numerous shoots coming from most lignotubers (Figure 4); shoots had reached an average height of 110 cm by November 2021. Prior to the fire, the main threat to the population seemed to be competition from *Leptospermum scoparium* but an adverse effect from *Phytophthora cinnamomi*, which is present on the site, cannot be ruled out. Basal resprouts prior to the fire generally did not survive, possibly because of the intense competition. There has been considerable regeneration of *Leptospermum scoparium* since the fire but of more concern is the large number of seedlings of *Eucalyptus sieberi*, which have appeared. If they reach maturity, the structure of the community will be greatly altered.

Figure 4. A resprouting plant of *Eucalyptus imlayensis*, 23 months after the fire of 2019 / 20; multiple shoots arise from the large lignotuber wedged into fractured rock on the steep eastern slope.

There have been two attempts at enhancement planting in the summit area, in September 2011 and October 2018. On each occasion, 20 plants were grown from seed and planted in a range of habitats close to the summit (but not within the boundary of the existing population, where there was insufficient soil to plant seedlings). Growth was slow but 36 of the 40 plants were alive in October 2019, ranging in height from 14 cm to 170 cm tall, though none had flowered in that time. Plants were tallest where competition was least. All 36 plants were killed by the 2019 / 20 fire. The time to reproductive maturity for this species is unknown but, from seed produced plants in the wild, it is clearly more than 10 years. Three plants in the ANBG which were grown from seed in 2007 have yet to flower, so it takes more than 15 years to first flowering in cultivation.

It may be a decade or more until the resprouting plants produce seed and, if similar to pre-fire seed production, few genets will produce seed at any one time and seed production will be minimal. The development of a seed orchard at the ANBG is the goal of a current project between DPE and the ANBG. This would allow for the collection and storage of pollen from flowers of all genets when these appear, and controlled hand pollination to aid seed production. Grafting trials will be undertaken with the aim of replicating the 17 genets in an ex-situ collection. If grafting is unsuccessful, the production of plants using tissue culture techniques will be attempted as insurance against loss of the wild population.

As a last resort, some of the small quantity of seed currently in conservation storage may be used to grow plants for ex-situ seed production.

Hibbertia circinata

Status: EPBC Act – critically endangered; BC Act – critically endangered; FFG Act – not relevant

Population size: pre-fire c. 200, post-fire 1000s but rapidly declining

Susceptibility to Phytophthora cinnamomi: highly susceptible

Fire response: obligate seeder (killed by fire)

Hibbertia circinata is a shrub growing to about 1.5 m tall with woolly stems and coiled hairs on its leaves (Figure 5a); it was recently separated from other taxa within *Hibbertia linearis* sens. lat. (McDougall et al. 2018). This species is endemic to the summit area of Mount Imlay, where it is co-extensive with *Boronia imlayensis*. Like *Boronia imlayensis* it is an obligate seeder and all of its mature population was killed by the fire of 2019 / 20. Seedlings were observed in May 2020, five months after the fire, and the population increased greatly compared with the pre-fire population of about 200 plants, appearing in parts of the summit where it had not been seen pre-fire. Up to 10% of the population was in flower in spring 2021. In glasshouse trials, this species was found to be highly susceptible to *Phytophthora cinnamomi* (McDougall and Liew, unpublished data), and deaths in the wild population have been numerous (Figure 5b). Indeed, no live plants were detected in some 4 m^2 monitoring plots within five months of the first measurement.

Hibbertia circinata has been successfully grown from cuttings at the ANBG and a small live population is maintained there and at BBG. There is no seed in storage, and the seed storage requirements and germination cues for the species are unknown.

The prognosis for the wild population of this species appears to be poor despite the large number of plants currently present. It might be maintained on Mount Imlay by using *Phytophthora*-free refugia and spraying with the chemical phosphite. Before the fire, plants grew most vigorously beneath the cliffs of the mountain; these may prove to be pathogen-free refugia because they don't seem to receive run-off from infested areas. Phosphite (an anionic form of phosphonic acid) has been shown to provide protection for some species and vegetation affected by *Phytophthora cinnamomi* (Barrett and Rathbone 2018) and has recently been applied in screening trials to *Hibbertia circinata* plants in the wild and in a glasshouse at the Royal Botanic Gardens Sydney. Plants in the glasshouse showed minor phytotoxicity but possible beneficial effects are still being assessed.

Figure 5. *Hibbertia circinata* a) grown from cuttings at ANBG, b) affected by *P. cinnamomi* on Mt. Imlay.

Olearia rugosa subsp. *distalilobata*

Status: EPBC Act – currently being assessed for listing as endangered; BC Act – not listed; FFG Act – not listed

Population size: pre-fire c. 250, post-fire 85 (in August 2021)

Susceptibility to Phytophthora cinnamomi: unknown

Fire response: resprouted after fire (and no seedlings observed)

Olearia rugosa subsp. *distalilobata* is a shrub that grows to about 1.5 m tall and produces corymbs of flowers in spring (Figure 6). It has been recorded in eastern Victoria but the only population in NSW is in a north-facing gully on a western ridge of Mount Imlay. Other gullies on the mountain with similar aspect were searched in October 2019 but no further populations were located.

All plants on Mount Imlay were burnt in the 2019 / 20 fire but resprouting was noted five months afterwards. The population is confined to an area of about 500 m^2. Although there are no obvious threats to the species on the mountain, numbers have declined since it was first documented there in 2015. The subspecies has also been recorded at seven sites in Victoria but only four of those were located during searches in 2007 (Andre Messina, Royal Botanic Gardens, pers. comm.). The total population size for all known sites was thought to be less than 500 plants prior to the 2019 / 20 fires, which also burnt much of its habitat in Victoria. The susceptibility of this taxon to *Phytophthora cinnamomi* is unknown but few daisies are known to be highly susceptible (Weste 2001); glasshouse testing of its susceptibility is recommended. *Olearia rugosa* subsp. *distalilobata* will probably be eligible for listing as endangered under the criteria used for the EPBC Act (area of occupancy, population size and fluctuations in populations) and is currently being considered for such a listing (https://www.dcceew.gov.au/environment/biodiversity/threatened/assessments/fpal).

Cuttings were taken from plants in 2016 and 2019 and have been grown at the ANBG. The subspecies can reliably be propagated from cuttings but no seed is held in conservation seed banks, and the requirements for seed storage and germination are not known. Given the very small area in which

the subspecies grows on Mount Imlay, the establishment of more populations elsewhere on the mountain will be useful to spread the risk of local extinction.

Figure 6. *Olearia rugosa* subsp. *distalilobata* on Mount Imlay in October 2015.

Persoonia brevifolia

Status: EPBC Act - not listed; BC Act – not listed; FFG Act - critically endangered

Population size: few plants, but possibly overlooked when not in flower

Susceptibility to Phytophthora cinnamomi: unknown

Fire response: unknown

This shrub, which may grow up to 1.8 m tall, has only been seen twice by the authors in the past 23 years on Mount Imlay, and then only as plants no more than 20 cm tall. From herbarium collections, there appear to be just three populations: Mt Imlay, Nalbaugh Plateau (both in NSW) and near Yambulla Creek in the Genoa River catchment in Victoria. There have only been two collections this Century (both on Mount Imlay). Collection notes for the Imlay and Yambulla populations indicate that the species is uncommon. On the Nalbaugh Plateau it is described as occasional or common. All three locations were burnt in the 2019 / 20 fires. The current population size is unknown but clearly small on Mount Imlay. Further survey of known locations (especially in early summer when the species is in flower) is urgently needed to assess post-fire recruitment and allow an assessment of conservation status.

The susceptibility of this species to *Phytophthora cinnamomi* is unknown but some species of *Persoonia* are highly susceptible (McDougall 2006). The rarity on Mount Imlay and the lack of tall individuals is suspicious of a species that is readily killed by this pathogen but that recruits occasionally from soil-stored seed. Like other *Persoonia* species, *Persoonia brevifolia* is extremely difficult to propagate from cuttings or from seed. No seed is held in conservation seed banks, and seed storage and germination requirements are not understood.

Philotheca virgata

Status: EPBC Act - not listed; BC Act – not listed; FFG Act - endangered

Population size: < 1000 plants pre-fire and post-fire

Susceptibility to Phytophthora cinnamomi: susceptible

Fire response: obligate seeder (killed by fire)

In NSW, *Philotheca virgata* is known only from Mount Imlay and the Nullica area to the north, where it is apparently rare. There are several hundred plants on Mount Imlay in two sub-populations, both of which are mostly *Phytophthora*-free. There may be more plants post-fire but the increase has not been as dramatic as with species such as *Hibbertia circinata*. Plants grow to about 1.5 m tall (and occasionally to 2m tall in the *Leptospermum scoparium* shrubland on the eastern slope). In Victoria, the species is found at only two sites in East Gippsland. It is widespread and common in western Tasmania and unlikely to be eligible for listing as threatened throughout Australia. However, Rozefelds (2001) notes morphological differences between populations and so it is possible that the mainland and Tasmanian populations will ultimately be separated taxonomically. As genetic and geographic outliers, the mainland populations are undoubtedly significant regardless, and worthy of protection. *Phytophthora cinnamomi* was recovered from the roots of symptomatic plants on Mount Imlay in early 2022; many plants were symptomatic at that time and so the species is possibly highly susceptible and at risk on Mount Imlay.

Whilst *Philotheca virgata* can be propagated with moderate success from cuttings, any conservation efforts will be impacted by the lack of any seed in conservation seed banks and information on how to store or germinate seed.

Prostanthera walteri

Status: EPBC Act - not listed; BC Act – not listed; FFG Act - endangered

Population size: pre-fire < 100, post-fire < 1000

Susceptibility to Phytophthora cinnamomi: highly susceptible

Fire response: obligate seeder (killed by fire)

This sprawling shrub is, like *Persoonia brevifolia*, known in NSW only from Mount Imlay and the Nalbaugh Plateau. In Victoria it has been recorded at several sites in East Gippsland.

On Mount Imlay it occurs mostly in the *Leptospermum scoparium* shrubland of the eastern slope below the summit ridge. The species was rarely seen pre-fire but more common after the fire. It appears to be highly susceptible to *Phytophthora cinnamomi* with many symptomatic plants observed in early 2022, and the pathogen was readily recovered from roots. The fact that this species was largely present in areas containing the pathogen before the fire and that it is susceptible, suggests that some susceptible species like this may be able to persist in low numbers despite the pathogen. Disease escapes are not unexpected in the rocky habitat where there is little soil and run-off is unlikely to reach all parts of the slope. Periodic seed production and long soil seed storage could enable some susceptible species to survive in this habitat. Given that this species is regarded as endangered in Victoria, where it occurs at more locations, it may be eligible for listing under the EPBC Act as endangered in Australia. Much of its habitat was burnt in the 2019 / 20 fires.

Whilst this species can be reliably propagated from cuttings, like other threatened species on the mountain its conservation is impacted by the lack of stored seed in seed banks, and knowledge about how to successfully store or germinate seed.

Tetratheca subaphylla

Status: EPBC Act - not listed; BC Act – not listed; FFG Act - vulnerable

Population size: < 1000

Susceptibility to Phytophthora cinnamomi: highly susceptible

Fire response: unknown

Tetratheca subaphylla is cryptic when not in flower, with its leaves reduced to small scales on narrow stems, making the plant look grass-like (Figure 7). This species was one of the first to disappear from the northern slopes of Mount Imlay after the arrival of *Phytophthora cinnamomi*. It is highly susceptible to infection, rapidly killed and apparently did not recolonise infested areas. The species has been recorded at several sites in the hinterland of the south coast of NSW (though, has not been relocated in post-fire surveys by the Royal Botanic Gardens Victoria), the western side of Kosciuszko National Park (where rare) and East Gippsland. It is regarded as vulnerable in Victoria and may be eligible for such a listing nationally if adequate data were available for its assessment. On Mount Imlay at least, while the species has re-appeared post-fire, there seems a chance that it will approach extinction in the coming decades.

Figure 7. Though cryptic when sterile because of its grass-like leaves, *Tetratheca subaphylla* is unmistakable when in flower. Photo: Andre Messina, CC BY-NC-SA 4.0 (https://creativecommons.org/licenses/by-nc-sa/4.0/).

The species can be reliably propagated from cuttings, but again lacks any stored seed in conservation seed banks and information about the storage or germination of seed.

Other species of note

There is a record of *Calotis glandulosa* (vulnerable under the BC Act and EPBC Act) in the Atlas of Living Australia, which we suspect is a data entry mistake. There is no vouchered specimen that can be checked and, based on where this species typically grows (i.e. grasslands and open woodlands of the southern and central tablelands), it is improbable in Mount Imlay National Park where there is no suitable habitat.

A few species are at or close to the edge of their range (e.g. *Leptospermum scoparium* and *Pseuderanthemum variabile*) on Mount Imlay and so their populations may have genetic significance for the species. *Pultenaea gunnii* was collected by von Mueller in 1860 from 'Barren ranges near Mount Imlay' (Corrick 1993) and may occur there. There are numerous records of this species in Victoria close to the NSW border. It is regarded as being highly susceptible to *Phytophthora cinnamomi* (Weste 2001) and might be listed as critically endangered in NSW if relocated.

Further taxonomic discoveries are possible on Mount Imlay, which may increase the conservation significance of the area. Until recently, *Boronia imlayensis* and *Hibbertia circinata* were included with more widespread taxa (*Boronia muelleri* and *Hibbertia linearis*). The same may be true for populations of *Epacris microphylla* and *Oxylobium ellipticum* on the summit ridge of Mount Imlay, which are superficially dissimilar to typical plants of these species. Shrubs of *Epacris microphylla* on Mount Imlay were up to 3 m tall but were all killed by the fire. This species is typically less than 1 m tall elsewhere in south-eastern Australia. The population of *Oxylobium ellipticum* on Mount Imlay has dense terminal floral racemes and leathery foliage unlike its low growing Alps form of the same name. Both are worth further taxonomic investigation.

Conclusion

Curiously, many species growing in the summit area of Mount Imlay are obligate seeders, including the dominant tree of the sheltered eastern slopes, *Eucalyptus fraxinoides*. This suggests that fire is an infrequent visitor to the mountain or at least to the sheltered parts of the mountain that are normally moist in summer. If fire frequency increases greatly because of climate change then local extinction seems inevitable for many of the significant species found there. That said, rare fire may well be important for *Eucalyptus imlayensis* by reducing competition from the dominant shrub *Leptospermum scoparium*. *Phytophthora cinnamomi* has increased pressure on some species, which are likely to be pushed into small refugia on the mountain in the coming years. It is perhaps unsurprising that most of the species we discuss above are restricted to areas where *Phytophthora cinnamomi* is apparently absent (based on soil sampling and the presence of the highly susceptible *Xanthorrhoea australis*).

Many of the significant species of Mount Imlay are likely to be eligible for listing as threatened under State and Commonwealth legislation, and the area itself will warrant listing as an Area of Outstanding Biodiversity Value under Part 3 of the *NSW Biodiversity Conservation Act 2016*, having multiple threatened species, including three species of global significance, and irreplaceable biological distinctiveness (Department of Planning, Industry and Environment 2021). However, the listing process can be time-consuming and for a species like *Persoonia brevifolia*, will require additional survey to assess population size at sites where it occurs. In addition, funding is not guaranteed even when species and areas are listed in legislation. On Mount Imlay, it may not be essential to have all species listed in order to undertake effective recovery measures because action on the main threat, *Phytophthora cinnamomi*, for one species will benefit many species. Similarly, the effort required to obtain seed or cuttings for germplasm storage for one species will not be greatly different to that for all species. The highest priority actions are seed collection and *Phytophthora* mitigation. The latter action is likely to involve spraying of the summit ridge with phosphite, which will probably be most cost-effective if applied by helicopter. Surveys are also needed to assess the significance of other life forms on Mount Imlay (e.g. fungi, insects, vertebrates) – it would be surprising if endemism and disjunction were a feature of only vascular plants.

Is Mount Imlay unusual in its number of endemics and share of locally rare species? Perhaps not; the region has many highly localised endemic plant species, some on mountain tops (e.g. *Nematolepis rhytidophylla* on Nalbaugh Plateau, *Acacia constablei* on Narrabarba Hill) and others on localised geological features (e.g. *Olearia astroloba* on limestone in East Gippsland and *Zieria buxijugum* on a rhyolite outcrop near Pambula). Indeed, the closest relative to *Eucalyptus imlayensis*, *Eucalyptus elaeophloia*, occurs only on Brumby Point, part of the high elevation Nunniong Plateau in East Gippsland, with many other highly localised plant species (Department of Sustainability, Environment, Water, Population and Communities 2011). Isolated plant populations may occur through speciation where long periods of isolation promote genetic change, rare dispersal events leading to disjunction, or fragmentation where habitat for a species was once widespread but becomes isolated through broad-scale environmental change (e.g. Raven 1972, Muellner-Riehl et al. 2019). At almost 900 m a.s.l., Mount Imlay is palpably isolated from other mountains; the nearest summit above 800 m being Egans Peak, almost 20 km to the north. Mount Imlay and many other peaks in south-eastern Australia have clearly been isolated for a long time, potentially facilitating speciation. However, climatic fragmentation may have contributed to species disjunctions in some cases. The Nunniong Plateau for instance shares several of its cool climate rare species with the Kosciuszko area.

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Reproductive biology of rainforest Rutaceae: floral biology, breeding systems and pollination vectors of *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*

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Abstract: The conservation of plant species requires an understanding of the factors that affect viable seed production, but often these factors are poorly understood. We investigated the reproductive biology of two Australian endemic rainforest species, *Acronychia oblongifolia* (A.Cunn. ex Hook.) Endl. ex Heynh and *Sarcomelicope simplicifolia* (Endl.) T.G.Hartley subsp. *simplicifolia*, with the intent of improving conservation and restoration outcomes.

The floral biology of these species was quantified to provide baseline data and insights into their pollination syndrome. Flower visitor surveys (using both digital recordings and human observations), a manipulative wind pollination experiment, and hand-pollination experiments were carried out to investigate pollination vectors and determine the breeding system.

Acronychia oblongifolia and *Sarcomelicope simplicifolia* subsp. *simplicifolia* were both found to best fit the general entomophily pollination syndrome. All floral visitors were arthropod species (*Acronychia oblongifolia*: 31; Sarcomelicope *simplicifolia* subsp. *simplicifolia*: 47) and fewer than 30% of the floral visitors identified, predominantly Diptera, Hymenoptera and Coleoptera, were regarded as potential pollinators. Failure of simulated wind gusts (40 km h^{-1}) to transport pollen 50 cm indicated anemophily is unlikely for these species. Autonomous and manipulative selfing treatments produced few (*Acronychia oblongifolia*: <3%) or no (*Sarcomelicope simplicifolia* subsp. *simplicifolia*) viable seed, indicating these are predominantly outcrossing species, although fruit and viable seed production were highly variable within and among all other treatments (open to natural pollinators, pollinator exclusion, pollinator exclusion and manipulative outcross, and pollinator exclusion and manipulative selfing). Pre-dispersal seed predation was recorded for both species, at several study sites. Pre-dispersal seed predation and increased distances between compatible individuals caused by habitat fragmentation, are two factors limiting the production of viable seeds for both species.

Key words: Viable seed, entomophily, entomophilous, White Aspen, Bauerella, Yellow-wood, Hard Aspen.

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Introduction

Viable seed contains a live embryo that can germinate under appropriate conditions (Bradbeer 1988), and therefore, the reproduction of non-clonal angiosperms depends on the production of viable seed. Many factors, both biotic and abiotic, affect seed set, seed development and seed maturation (Burd 1994; Fenner & Thompson 2005; Chen & Zuo 2019). As a result, viable seed production in angiosperms is highly variable. Some common hypotheses for the factors that prevent viable seed set include inbreeding (Baskin & Baskin 2015), pollen limitation (Stephenson 1981), resource limitation (Lee 1988) and seed predation (Auld 2001; Armstrong 2002).

Over 85% of angiosperms require a biotic pollination vector for outcross seed set (Ollerton *et al.* 2010). Species with variable or poor fruit production, and in turn seed set, are often hypothesized to be pollen limited (Stephenson 1981; Knight *et al.* 2005; Chen & Zuo 2019). An inadequate supply of viable and compatible pollen grains, or a low abundance of biotic pollinators, can cause pollen limitation (Chen & Zuo 2019). However, for many species the ecological interactions needed for seed production are unknown; this includes whether a pollination vector is needed to set seed and, if so, what the pollination vector is (Williams & Adam 2001; Bennet *et al.* 2018). This significant gap in our understanding of the factors that affect viable seed production has impeded conservation and restoration for some angiosperm species (Martyn *et al.* 2009).

Viable seed production is known to be variable in some species, particularly Rutaceae (Auld 2001; Martyn *et al.* 2009). Rutaceae are common and widespread in rainforests of eastern Australia (Mills & Jakeman 1995); however, these rainforests in the Illawarra landscape are now highly fragmented communities, as a result of post-European settlement (Mills 1988; Mills & Jakeman 1995), particularly clearing for agriculture. *Ex situ* conservation is vital to provide insurance against extinction for individual species and populations, and to support *in situ* management and restoration (Sommerville *et al.* 2017). Seed banking is considered the most effective *ex situ* conservation technique (Offord & Meagher 2009); however, seed banking hinges on sourcing and storing viable seed (Martyn Yenson *et al.* 2021). Nevertheless, the factors influencing viable seed production in rainforest Rutaceae are rarely examined (Auld 2001; Armstrong 2002; Martyn *et al.* 2009).

Acronychia oblongifolia and *Sarcomelicope simplicifolia* subsp. *simplicifolia* are Rutaceae species that inhabit temperate and subtropical rainforests of eastern Australia (DPIE 2019a; DPIE 2019b). Both have wide coastal distributions, *Acronychia oblongifolia* from Queensland to Victoria, and *Sarcomelicope simplicifolia* subsp. *simplicifolia* from North Queensland to Mt Dromedary on the NSW South Coast. Seed set is known to be variable in both species, but the causes of this remain unknown (Martyn *et al.* 2009). *Sarcomelicope simplicifolia* subsp. *simplicifolia* is a dioecious tree (Pellow *et al.* 2011), while *Acronychia oblongifolia* produces hermaphroditic flowers (PlantNET 2020). No other aspects of the reproductive biology of these species have previously been studied.

The aim of this study was to investigate the floral biology, pollination vectors and breeding systems to determine the factors contributing to seed production in these two common rainforest species. Specifically, this study aimed to: 1) examine the floral morphology and phenology of *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* to provide insights into the likely pollination syndrome and breeding system; 2) identify floral visitors and, by analysing the foraging behaviour and pollen load of those visitors, identify the likely pollinators; 3) determine whether anemophily plays a role in pollination of these species; 4) confirm the breeding system by quantifying fruit and seed set in response to hand pollination experiments.

Methods

Study sites

This study was conducted between March 2020 and May 2021 on plant populations located in remnant rainforest, restored rainforest and botanic gardens mainly in the Illawarra area south-eastern New South Wales (NSW), Australia. *Acronychia oblongifolia* trees were located at two sites: one restored population at Jerrara Dam Reserve (34°40′19″S, 150°48′22″E) (hereafter Jerrara) and one planted population at the Australian Botanic Garden, Mount Annan (34°04′14″S, 150°46′04″E) (hereafter Mount Annan). The original provenance of the trees at Mount Annan was Boatharbour Nature Reserve, New South Wales. *Sarcomelicope simplicifolia* subsp. *simplicifolia* trees were studied at four sites: three restored populations at Jerrara, Spring Creek Wetland Kiama (34°39′41″S, 150°50′48″E) (hereafter Kiama) and Spring Creek Wetland South Kiama (34°39′48″S, 150°50′40″E) (hereafter South Kiama); and one planted population at Wollongong Botanic Garden (34°24′34″S, 150°52′30″E) (hereafter Wollongong). The trees planted at Wollongong were sourced from natural populations in the Illawarra region (Carl Glaister pers. comm.).

The methods for studying the floral biology, floral visitors and pollination vectors, and the breeding systems followed those described in Lopresti *et al.* (Under Review).

Floral biology

To examine the floral phenology of each species, 20-40 flowers from 2-4 trees were observed daily from floral opening to abscission or fertilisation (following Kearns & Inouye 1993). On each day of observation, the anthers and stigmas of each flower were classed as immature, mature or senesced. The anthers were considered mature when pollen grains were visible when viewed through a hand lens. Stigma maturity was based on the size and colour of the stigma. Stigma receptivity was confirmed by a hydrogen peroxide test (following Kearns & Inouye 1993). For each species, 10 flowers each from individual plants thought

to contain mature stigmas were placed in a petri dish and individually examined under a 40 x dissecting microscope. Hydrogen peroxide (3% v/v) was dropped onto each stigma and observed for 60 seconds. The formation of bubbles on the stigma was considered an indication of receptivity. The first day that the androecium (male) and gynoecium (female) appeared mature, the duration of maturity, and the total anthesis period were recorded for each species.

Pollen morphology and exine characteristics were described as outlined by Halbritter *et al.* (2018) to gain insight into potential pollination vectors, and an indication of breeding system. Pollen characteristics described included: the shape (both equatorial (E) and polar (P) views); the length of the polar axis compared to the equatorial diameter (the P/E ratio); the dispersal unit of pollen grains; and the ornamentation on the aperture and exine regions. Stamens from three flowers of each target species were examined under a scanning electron microscope (JEOL JSM-6490LA, Japan) on the day of collection (*Acronychia oblongifolia*) or following a brief period of refrigerated storage (*Sarcomelicope simplicifolia* subsp. *simplicifolia*). Samples were mounted on a metal stub using conductive double-sided carbon tape and sputter coated with 20 nm gold using an Edwards AUTO 306 Sputter Coater (Edwards Australia, Yatala, Queensland). Imaging was completed in high vacuum mode at 10 mm working distance using SEI/BSE imaging at 15 KV operating voltage and with a spot size setting of 45.

Nectar production was measured to determine whether flowers provided rewards to potential pollinators. Prior to quantifying nectar production, inflorescences that housed at least five buds near to opening were selected haphazardly and bagged using a hard plastic inner layer of coarse mesh (Saxon Gutter Guard) covered with polyorganza fabric (15 cm x 35 cm) to prevent biotic visitors extracting nectar prior to the flowers opening. For *Acronychia oblongifolia*, nectar was extracted from 10 flowers with a mature androecium and 10 flowers with a mature gynoecium at Mount Annan. For *Sarcomelicope simplicifolia* subsp. *simplicifolia*, nectar was extracted from five flowers with a mature androecium from staminate trees, and five flowers with a mature gynoecium from pistillate trees, at each of Wollongong, Jerrara, Kiama and South Kiama. Nectar was extracted into a 1 µl or 5 µl microcapillary tube and the volume withdrawn was determined by calculating the proportion of the column that was filled (in accordance with Morrant *et al.* 2009). Nectar produced in flowers with a mature androecium and a mature gynoecium were compared using a t-test on untransformed data (data were normally distributed). Due to the small sample size, data were pooled across sites.

Floral visitors and biotic pollination vectors

Any fauna species that contacted a flower were regarded as floral visitors. A combination of diurnal and nocturnal human observations, video recordings, time lapse photography (TLP), and nocturnal infrared motion sensor imaging were used to observe floral visitors. Sampling was conducted on 2-6 trees at 2-4 sites for each species (Table S1 details the survey effort). Observations were done on sunny days with no or light wind speeds (gusts did not exceed 20 km h^{-1}), and temperatures ranged between 17°C and 32°C.

Diurnal human observations and video recordings were conducted during the day (10:00-15:00). TLP was conducted from first light to last light, to capture species that may forage outside the 10:00–15:00 period. Infrared cameras were recording between last light and first light, and nocturnal human observations were undertaken for three hours commencing after last light. Sampling was done on the outermost part of canopy containing high floral densities, and on flowers containing mature reproductive structures, for all sampling methods.

Human observations were done on six fixed sections (50 cm x 50 cm) of canopy on each tree. Floral visitors were opportunistically captured following their foraging bout and euthanized. Captured specimens were identified and their pollen load was analyzed under a stereo microscope (40 x magnification).

Digital video recordings (DVR, 1080p resolution) of inflorescences containing a high proportion of open flowers were obtained using GoPro Hero4 Session CHDHS-101 digital cameras (GoPro Australia Pty Ltd) attached to a tripod. Up to six DVRs were simultaneously recording for a two-hour period on each day that observations were done. Each camera had a 15 cm x 30 cm field of view.

Time-lapse photographs (TLP) were captured on Brinno TLC200 Pro HDR cameras (Brinno Incorporated, Taiwan). Over three non-consecutive days, three time-lapse cameras were set to capture an image every two seconds between sunrise and sunset (at four study sites). The field of view of each camera captured a 15 cm x 30 cm area of canopy. TLP was employed to guide the survey effort for DVR and diurnal human observations.

Nocturnal human observations were conducted over three non-consecutive nights at one (Jerrara: *Arconychia oblongifolia*) or three (Jerrara, Kiama, South Kiama: *Sarcomelicope simplicifolia* subsp. *simplifcifolia*) study sites. Sampling was conducted in one 3 h block each night, starting at last light. A battery-operated head torch was used to scan floral patches for 5 seconds (following Hermansen *et al.* 2014). Two observers scanned the canopy every 55 seconds, and each hour a new tree was surveyed. *Acronychia oblongifolia* trees flowered asynchronously, so scans were done on the most suitable tree each night (suitability based on individuals with high floral density). Nocturnal human observations were not carried out at Wollongong or Mount Annan due to evening access restrictions.

Nocturnal infrared camera recordings (ICR) were captured on Bushnell Trophy Cam HD Essential E3 Trial motion sensor cameras. Cameras were set to record a single inflorescence (camera field of view 15 cm x 30 cm) from last light to first light. Over three nights, three cameras were set at both Mount Annan and Jerrara for *Acronychia oblongifolia* sampling. For *Sarcomelicope simplicifolia* susbp. *simplicifolia*, two nocturnal cameras were set to record over two nights at each of the four study sites. ICR was employed to guide the survey effort for nocturnal human observations.

Floral visitors that either carried pollen of the target species on their body or contacted the stigma while foraging were classed as potential pollinators (following Kearns & Inouye 1993; Dafni 1992). To distinguish floral visitors from potential pollinators for species detected by DVR, contact with the stigma was recorded for all floral visitors. Pollen presence for captured individuals was examined under a stereo microscope (40 x magnification). Specimens carrying pollen were then examined under a scanning electron microscope (SEM) (JEOL JSM-6490LA, Japan) to examine whether the pollen was that of the target Rutaceae species. Specimens were mounted as previously described for scanning electron microscopy.

To determine whether there was a dominant pollinator taxon, the percentage of sampling intervals in which a potential pollinator of a given taxon was present (hereafter referred to as 'presence') was compared among species for each of the observational methods that a potential pollinator was detected by: human observations and DVR. Potential pollinator presence was standardized by the duration of the sampling interval (5 minutes for human observations and 2 hours for DVR). 'Presence' provided an estimate of the visitation frequency for each potential pollinator observed under each survey method.

To examine whether the presence differed among potential pollinators, a one-way analysis of variance (Kruskal and Wallis 1952) was applied for human observation and DVR data as normality and equal variance assumptions were not met for transformed data. A Dunn All Pairs for Joint Ranks test was undertaken when a significant difference arose. Data were pooled across sites for all of these analyses as pollinator species differed among sites. All data were analysed using the statistical software JMP Pro 15.

Wind pollination

To examine whether wind may be a pollination vector, the pedicels of flowers that contained visible pollen (viewed through a hand lens) were taped to a rod so that the anthers were exposed. A fan was used to generate wind speeds (5 km h^{-1} and 40 km h^{-1}) blowing onto each flower for 10 seconds. A 9 cm Petri dish smeared with Vaseline was placed behind each open flower to capture any dislodged pollen. Wind speeds were measured using a handheld anemometer (Kestrel® 3500, accuracy ± 3%) and were selected based on below and above average wind speeds within the Illawarra region (recorded at Bellambi AWS from 1997-2010, weather station ID 068228 , BOM 2023). To determine the maximum distance pollen could travel, petri dishes were placed at 5 cm, 10 cm or 50 cm behind the open flowers. This design yielded six wind speed x distance treatment combinations: (1) 40 km h^{-1} x 5 cm, (2) 40 km h^{-1} x 10 cm, (3) 40 km h^{-1} x 50 cm, (4) 5 km h^{-1} x 5 cm, (5) 5 km h^{-1} x 10 cm and (6) 5 km h^{-1} x 50 cm. Pollen traps for treatments one, two, four and five comprised a single Petri dish, whereas those for treatments three and six comprised four Petri dishes arranged in a square to increase the surface area available for pollen capture. To determine whether pollen grains were present on the traps, the pollen traps were observed under a light microscope (100 x magnification). Treatments were replicated 10 times each for *Acronychia oblongifolia*, using flowers sourced from one tree at Mount Annan and one tree at Jerrara, and 15 times each for *Sarcomelicope simplicifolia* subsp. *simplicifolia*, using flowers sourced from one tree at each of Jerrara, South Kiama and Kiama.

A two-factor ANOVA was conducted to determine the effect of wind speed and distance on the percentage of replicates bearing pollen. Treatments five and six were excluded from this analysis to maintain an orthogonal design, as there was no pollen on any plates for treatment five, and as a result treatment six was not applied. Data were arcsine-transformed to ensure homogeneity of variance and normality assumptions were met.

Breeding Systems

A series of pollination experiments were conducted for both species to determine their breeding systems. Inflorescences containing at least five buds were haphazardly selected and subjected to one of five randomly applied treatments: (1) manipulative outcross, (2) autonomous selfing, (3) manipulative selfing, (4) open control (no bag), or (5) procedural control (partial bag). Inflorescences were selected on the same day and open flowers, or very young buds were removed so that flowers developed synchronously within and among the treatments. Polyorganza bags (15 cm x 35 cm) were used to exclude floral visitors throughout the experiment.

Manipulative outcrossing involved bagging an inflorescence in the bud phase, and then hand pollinating each flower as the stigma became receptive, with pollen sourced from a donor tree located at least 10 m away but within the same population. Manipulative selfing was treated the same way, but with pollen sourced from the same tree. Both manipulative treatments were done over three consecutive days to maximise the likelihood of viable pollen being deposited on a receptive stigma. Autonomous selfing involved bagging an inflorescence in the bud phase without manipulating the flowers. Control inflorescences were open to natural pollinators and not manipulated. The procedural control had three openings cut into each bag (10 cm x 5 cm) which tested whether the presence of a bag affected experimental outcomes by allowing biotic pollinators to visit the flowers. Bags that had signs of insect activity, or where the bag tore during the experimental period were excluded from the analysis.

To hand pollinate *Acronychia oblongifolia* flowers, 8 to 16 filaments that held visible pollen (viewed through a hand lens) were removed with forceps from one or two flowers that were not assigned a pollination treatment. Anther heads were brushed onto the receptive stigma of each flower that was assigned a hand pollination treatment. To hand pollinate *Sarcomelicope simplicifolia* subsp. *simplicifolia*, two staminate flowers that were not assigned to a pollination treatment and held visible pollen (viewed through a hand lens) were removed and anthers were brushed onto each stigma that required hand pollination until pollen was visible on the stigma's surface.

Treatments were applied and replicated five (*Acronychia oblongifolia*) or ten (*Sarcomelicope simplicifolia* subsp. *simplicifolia*) times per tree. The reproductive success for all inflorescences was monitored, from pollination through to the maturation of fruit. Early fruit development was based on the ovary swelling, after the petals and stamens had senesced. Fruit maturity was based on the size and the colour of the fruits. Mature fruits were harvested and examined for viable seed. Reproductive success was classed as the percentage of flowers that developed fruit (early fruit development), and the quantity of viable seeds produced per treatment.

Fruits and seeds were dissected and examined under a stereo microscope (40x magnification) to determine the number of seeds per fruit and the proportion of filled seeds for all treatments. A 'filled' seed housed an embryo and endosperm filling the entire space and an 'empty' seed lacked both embryo and endosperm. A 'partially filled' seed contained an embryo, but the endosperm did not fill the entire space enclosed by the seed coat; these seeds are potentially viable. Seeds were also classed as 'not predated' or 'predated' based on signs of pre-dispersal seed predators (e.g., frass or an exit hole in the seed coat).

To determine the reproductive success for each species, the percentages of flowers that produced fruits were compared among treatments using a one-way ANOVA. For both plant species, data were pooled from all sites and trees, and arcsine transformed if needed. A Tukey HSD analysis was applied when a significant difference arose. For both plant species, a separate analysis was undertaken to examine whether spatial factors influenced fruit production; site was included as a factor and a subset of the data was used to ensure a balanced design. For *Acronychia oblongifolia*, a two-way ANOVA (tree, treatment) was conducted on the two plants at Jerrara that all treatments were applied to. For *Sarcomelicope simplicifolia* subsp. *simplicifolia*, a three-factor nested ANOVA was carried out on three individuals from each site. 'Plant' and 'site' were considered random factors and 'plant' was nested in 'site'. A t-test was undertaken to compare fruit set between the open control and procedural control to examine whether bagging affected the results for both species.

Results

Floral biology

The sequence of floral development and maturity of reproductive structures indicated *Acronychia oblongifolia* is protandrous and confirmed that *Sarcomelicope simplicifolia* subsp. *simplicifolia* is dioecious (Figure 1). For both species, the androecium was mature and usually dehisced within one day of floral opening. Pollen was dispersed on average 2 (± 0.07 SE) or 3 (± 0.05) days after floral opening for *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*, respectively. For *Sarcomelicope simplicifolia* subsp. *simplicifolia*, pistillate flowers contained a mature gynoecium upon opening and remained receptive for on average 5 (± 0.01) days; staminate flowers dehisced on average 4 (± 0.4) days after opening, while pistillate flowers dehisced on average 7 (± 0.5) days after opening. For *Acronychia oblongifolia*, the stigma became receptive on average 5 (± 0.1) days after floral opening and remained receptive for on average 4 (± 0.1) days. Pollen was dispersed prior to stigma receptivity in 17 of the 25 *Acronychia oblongifolia* flowers observed. The anthesis period was on average 12 (± 0.14) days.

Individual *Acronychia oblongifolia* trees flowered for approximately 6 weeks. Pistillate *Sarcomelicope simplicifolia* subsp. *simplicifolia* trees flowered for approximately 4 weeks, while staminate *Sarcomelicope simplicifolia* subsp. *simplicifolia* trees typically produced flowers for up to 9 weeks.

Figure 1. *Acronychia oblongifolia* flowers housing (a) mature androecium and immature gynoecium and (b) old androecium and mature gynoecium, and *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers showing (c) a staminate flower with mature stamens and (d) a pistillate flower with a receptive stigma. Scale bar = 1 mm.

Based on SEM observations, the external morphology of pollen grains across the plant species was indistinguishable, apart from variation in the grain size. All pollen grains were presented as discrete single units (monads) and were elliptical in overall shape. Based on the P/E ratio, the pollen shape was prolate. From polar view, the pollen grains were three-lobed circular and isopolar. Exine ornamentation was heterobrochate. Each grain contained three equidistant apertures with two distinct aperture types: endoaperture present (colporus aperture type) or endoaperture absent (colpus aperture type). Based on the size classes presented in PalDat (2020), *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* pollen grains may be considered 'medium' sized (approximately 40 µm and 30 µm diameters, respectively).

Acronychia oblongifolia flowers with a mature androecium produced significantly more nectar (twice the volume) than flowers with a mature gynoecium ($t_{(1, ,38)}$=5.740, p=0.028). Overall, the volume of nectar produced in *Acronychia oblongifolia* flowers did not exceed 0.23 µl. *Sarcomelicope*

simplicifolia subsp. *simplicifolia* staminate flowers produced significantly less nectar than pistillate flowers ($t_{(1, 39)}$=12.454, p=0.001). Overall, the volume of nectar produced in *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers did not exceed 0.4 µl (mean pistillate volume), while staminate flowers produced less than half that volume of nectar.

Floral visitors and biotic pollination vectors

Floral visitors were diverse (31 species for *Acronychia oblongifolia*; 47 species for *Sarcomelicope simplicifolia* subsp. *simplicifolia*) and consisted only of arthropods (Table S2). For *Acronychia oblongifolia*, Diptera was the most species rich taxon found visiting (20 species), followed by Coleoptera (4 species), Hymenoptera (3 species) and Lepidoptera (3 species). Species richness was greater at Jerrara (14 species) than Mount Annan (10 species), and only three species, all Diptera, were detected at both sites (Calliphoridae species 2, *Dichaetomyia* and *Melangyna* species). For *Sarcomelicope simplicifolia* subsp. *simplicifolia*, Diptera was the most species rich taxon found visiting (35 species), followed by Hymenoptera (9 species), Coleoptera (2 species), Hemiptera (1 species) and Arachnida (1 species). Species richness was greatest at Jerrara (28 species), and 19% of species were detected at more than one site. Four species were detected visiting *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers at three sites: *Calliphora* (species 2) and *Apis mellifera* Linnaeus, 1758 were both detected at Kiama, South Kiama and Jerrara, while the *Melangyna* species was detected at Kiama, South Kiama and Wollongong. Species richness of floral visitors was greatest at Jerrara (28 species), with more than double the number of species detected at any other site. No nocturnal visitors were detected by human observation or infrared video recording for either plant species.

Nine of the 31 floral visitors contacted the stigma or carried pollen and, therefore, are potential pollinators (hereafter referred to as pollinators) of *Acronychia oblongifolia* (Table S2). Across both sites, two species carried pollen and contacted the stigma as they foraged (the flies: *Dichaetomyia* species and an unidentified Calliphoridae). Additionally, four species contacted the stigma while foraging: a hoverfly (*Melangyna* species), a fly (*Pogonortalis* species), a beetle (*Luperini* species) and a butterfly (*Heteronympha mirifica* Butler, 1866). Three of the captured species carried pollen on their bodies: a fly (*Chrysomya rufifacies* Macquart, 1844) and two beetles (and an unidentified Elmidae and *Chauliognathus* species).

Eleven of the 47 floral visitors contacted the stigma or carried pollen of *Sarcomelicope simplicifolia* subsp. *simplicifolia* and therefore are potential pollinators (hereafter referred to as pollinators) (Table S2). Across all sites, two species contacted the stigma while foraging and carried pollen on their bodies: a bee (*Apis mellifera*) and a fly (*Calliphora* species 2). Additionally, two flies contacted the stigma while foraging (*Calliphora* species 3 and the unidentified species 31). Seven of the captured species carried pollen on their bodies: three flies (*Dichaetomyia* species, Muscidae species 9 and Tephritidae species 19), two native bees (*Exoneura* species and *Hylaeus* species), a beetle (Chrysomelid species 1) and an ant (Dolichoderinae species).

For *Acronychia oblongifolia*, the presence analysis (percentage of sampling intervals where a pollinator was present) revealed variable trends in visitation frequencies of pollinators across both sampling methods. Fly species of two taxa, *Dichaetomyia* and Calliphoridae, were the most frequent pollinators detected by human observations, present in significantly more sampling intervals than any other pollinator (x^2_5 =127.772, p<0.0001) (Figure 2a). These fly species were observed in 27% of sampling intervals, while all other pollinators detected by human observations were present in fewer than 2% of sampling intervals. Two flies, *Dichaetomyia* and *Pogonortalis* species, and a beetle, *Luperini* species, were all present in the greatest number of sampling intervals detected on DVR (16%) (Figure 2b); although pollinator presence detected on DVR did not significantly differ among species (x^2_5=3.817, p=0.576). TLP revealed few floral visitors foraged outside the 10:00-15:00 survey period (Figure S1) and all floral visitors were Dipteran species.

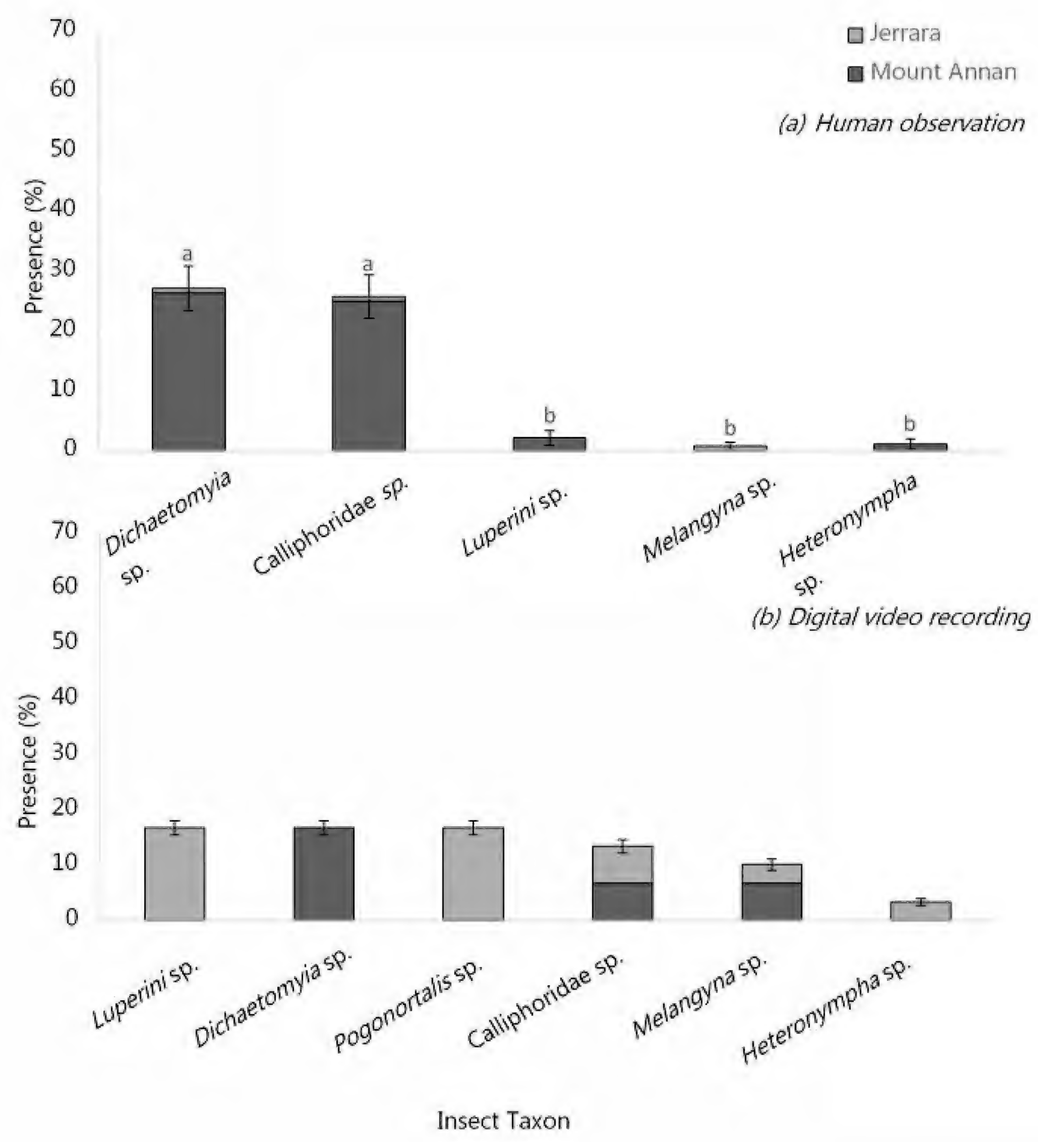

Figure 2. Potential pollinators of *Acronychia oblongifolia* as detected by (a) human observations for 5-minute intervals (n=144), and (b) digital video recordings for 2 hours (n=30). Note that different methods detected different pollinators. Data presented are means ± SE; bars with different letters are significantly different, according to Dunn All Pairs for Joint Ranks test after Kruskal-Wallis analysis.

The presence analysis of *Sarcomelicope simplicifolia* subsp. *simplicifolia* pollinators revealed the fly, *Calliphora* (species 2), and the bee, *Apis mellifera,* to be the most frequent floral visitors across both sampling methods. Human observations showed *Calliphora* (species 2) to be detected in a significantly greater number of sampling intervals than any other taxa (18%) (x^2_{10}=213.112, p<0.001; Figure 3a). *Calliphora*

(species 2) was also the most frequent floral visitor detected by DVR; present in significantly more sampling intervals than the other Dipteran species (species 31 and Muscidae species 9) ($F_{(3, 108)}$=5.648, p=0.001; Figure 3b). All other pollinators were infrequent floral visitors, present in less than 8% of sampling intervals, for both sampling methods (Figure 3). TLP detected some floral visitors active outside the 10:00-15:00 survey period but the suite of species did not differ; nine Dipterans and one bee (*Apis mellifera*) were recorded foraging earlier in the morning or later in the afternoon (active between 07:40 and 16:15) (Figure S2).

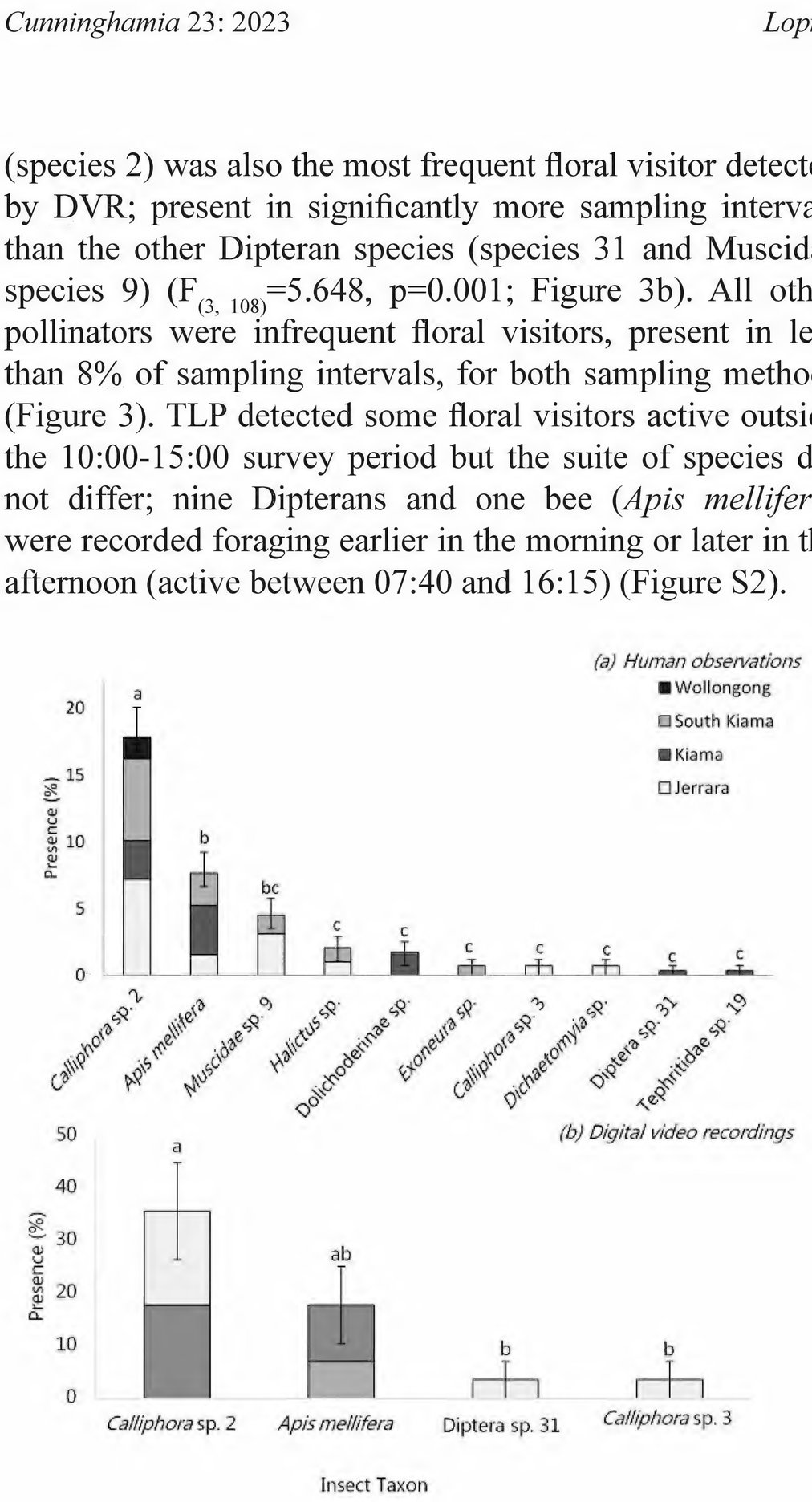

Figure 3. Potential pollinators of *Sarcomelicope simplicifolia* subsp. *simplicifolia*, as detected by (a) human observations for 5 minute intervals (n=288), and (b) digital video recordings for 2 hours (n=28). Note that different methods detected different pollinators. Data presented are means (± SE); bars with different letters are significantly different, according to (a) Dunn All Pairs for Joint Ranks test or (b) Tukey HSD analysis, after Kruksal-Wallis analysis or ANOVA, respectively.

Wind pollination

No pollen grains were detected on the *Acronychia oblongifolia* pollen traps, regardless of windspeed or plate distance; indeed, through visual inspection there were no signs that pollen had been dislodged from the anthers during the experiment.

Wind dislodged pollen of *Sarcomelicope simplicifolia* subsp. *simplicifolia*, but the grains did not travel far (≤10 cm) (Figure 4). Pollen presence differed significantly among some treatments ($F_{(3, 66)}$=3.611, p=0.018) as strong winds (40 km h^{-1}) dislodged pollen more frequently than light wind speeds (5 km h^{-1}) (Figure 4). Pollen was transported short distances (5 cm) in both strong (40 km h^{-1}) and light (5 km h^{-1}) winds, although only with strong wind was pollen dispersed further than 5 cm. Treatment six (5 km h^{-1} x 50 cm) was not applied due to time constraints and as no pollen was detected in treatments three or five (40 km h^{-1} x 50 cm and 5 km h^{-1} x 10 cm).

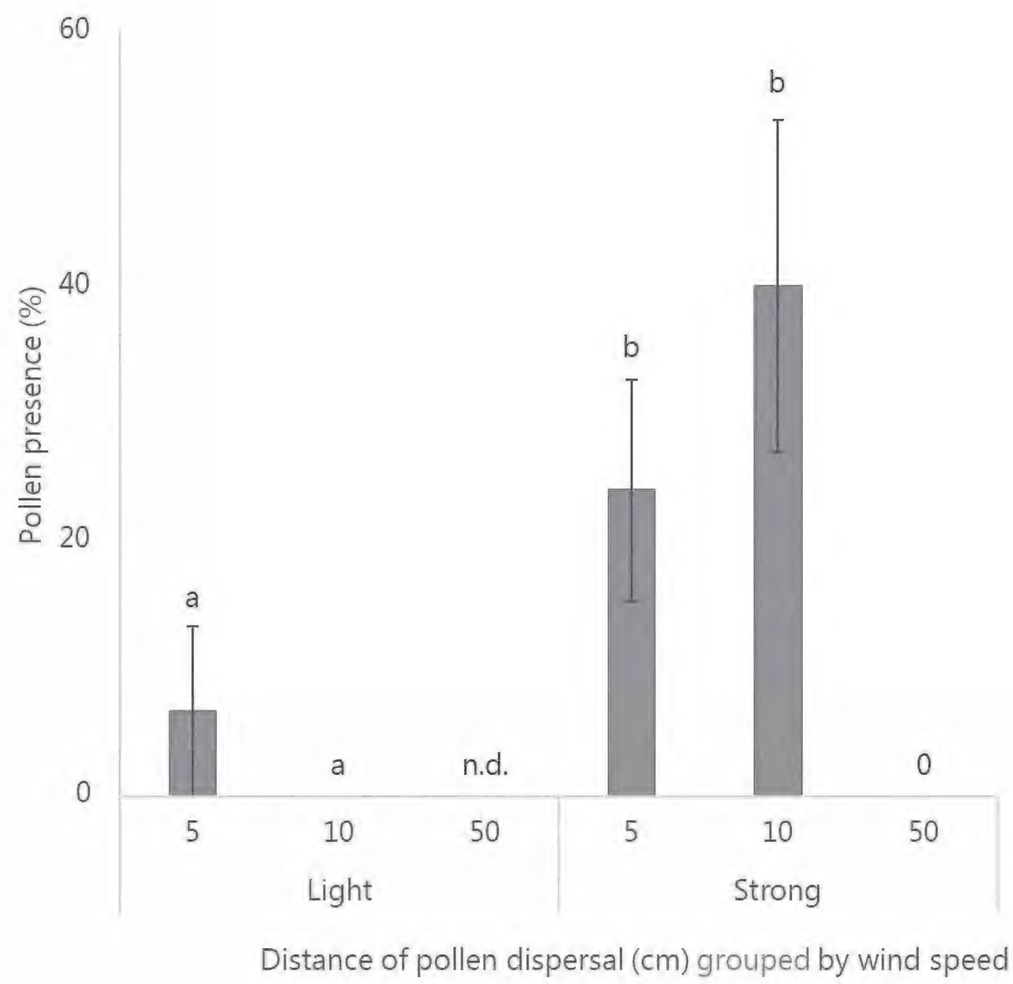

Figure 4. Mean (±SE) percent of plates with pollen of *Sarcomelicope simplicifolia* subsp. *simplicifolia* after exposure to one of six manipulative wind pollination experiments. Light (5 km h^{-1}) and strong (40 km h^{-1}) wind speeds were simulated, with pollen traps 5 cm, 10 cm or 50 cm behind one open flower. Bars with different letters are significantly different, according to two-way ANOVA. Note the 50 cm treatments were excluded from statistical analysis. n.d. indicates no data for that treatment.

Breeding systems

Fruit production did not differ significantly between the partial bag and open treatment with no bag for either plant species (*Acronychia oblongifolia*: $t_{(1, 27)}$=0.477, p = 0.496; *Sarcomelicope simplicifolia* subsp. *simplicifolia* $t_{(1, 81)}$=0.149, p = 0.701) indicating that bags have no substantial effect on fruit production and, therefore, open controls could be legitimately compared to treatments with bags.

Acronychia oblongifolia

Fruit was produced from all treatments except manipulative selfing; however less than 10% of flowers produced fruit overall (Figure 5). Fruit production varied significantly among treatments ($F_{(3, 121)}$=6.099, p=0.007; Figure 5a), with flowers that received experimental pollen from a different tree (manipulative outcross) producing more than four times as many fruits as those subjected to any other treatment (Figure 5a).

The balanced analysis comparing fruit set between two trees at Jerrara demonstrated that fruit set differed significantly between trees, but this was dependent on treatment ($F_{(3, 32)}$=3.743, p=0.002). Fruit production where pollen was sourced from a different tree (manipulative outcross) varied between trees, with flowers from tree three producing nearly ten times as much fruit as tree five (Figure 5b).

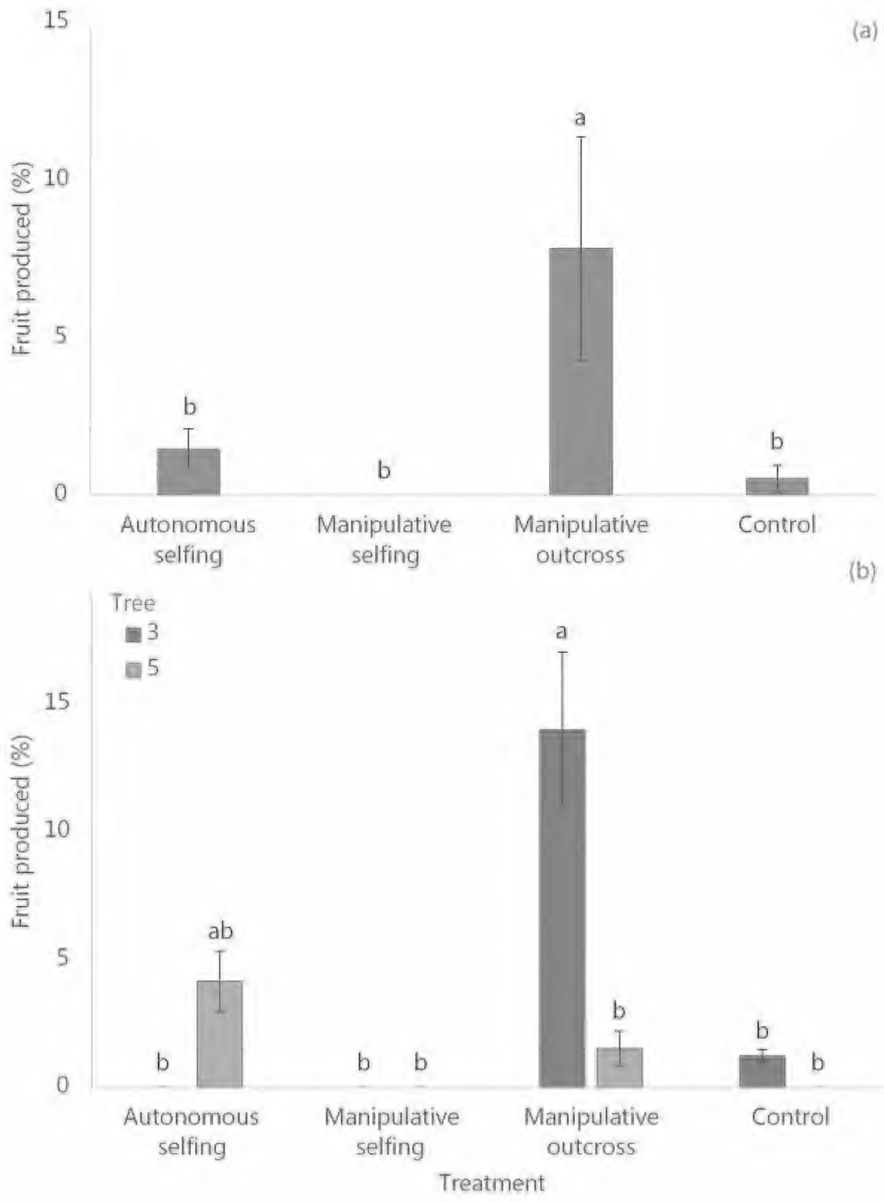

Figure 5. Mean (±SE) percentage of *Acronychia oblongifolia* flowers that produced fruit following pollination treatments: (a) data for seven plants and two sites are combined (autonomous selfing n=35; manipulative selfing n=20; manipulative outcross n=15; and open n=30); (b) plants located at Jerrara are included as a factor in a balanced design (n=5). For each panel, bars with difference letters are significantly different, according to Tukey HSD analysis after ANOVA.

Fruits and seeds were harvested from the outcross and autonomous selfing treatments, or no treatment, and of the seeds collected, 41%, 47% and 47% respectively were filled and therefore likely viable. Almost half (47%) of 19 seeds sourced from the autonomous selfing treatment were likely viable. However, this treatment also yielded the highest proportion of partially filled seeds (50%) compared with the outcross treatment or inflorescences that were not exposed to a treatment (13% and 2% respectively). Overall, 33% of 91 seeds were empty and not viable. Seeds from both Jerrara and Mount Annan were predated, with obvious small holes visible or frass within the seed, however <5% of seed had signs of insect predation.

Sarcomelicope simplicifolia subsp. *simplicifolia*

On average, 23% of flowers produced fruit, but fruit production varied significantly among treatments ($F_{(2, 250)}$=38.777, p<0.001; Figure 6a). Flowers that received experimental pollen from a different tree (manipulative outcross) produced the greatest abundance of fruit, more than double that of any other treatment (Figure 6a). Nearly 20% of flowers exposed to natural pollinators (open control) produced fruit, while no fruit was produced when pollinators were excluded from flowers (Figure 6a).

On average, more fruit was produced at Kiama (27%) than Wollongong (10%), but fruit production was highly variable among trees at both sites, and these differences depended on treatments (Table 1; Figure 6b). Trees one and three produced six times more fruit than tree two at Kiama; and significantly more fruit was produced on trees one and three at Kiama than any tree at Wollongong (Figure 6b). Fruit set in flowers exposed to natural pollinators (open control) was also highly variable among trees and between the two sites. The trees at Wollongong all produced fruit in low quantities, with less than 10% of flowers fruiting, while more than 60% of flowers on tree three at Kiama fruited (Figure 6b). Importantly, outcomes were consistent in terms of the source of pollen, with no fruit set on any tree when pollinators were excluded from the flowers.

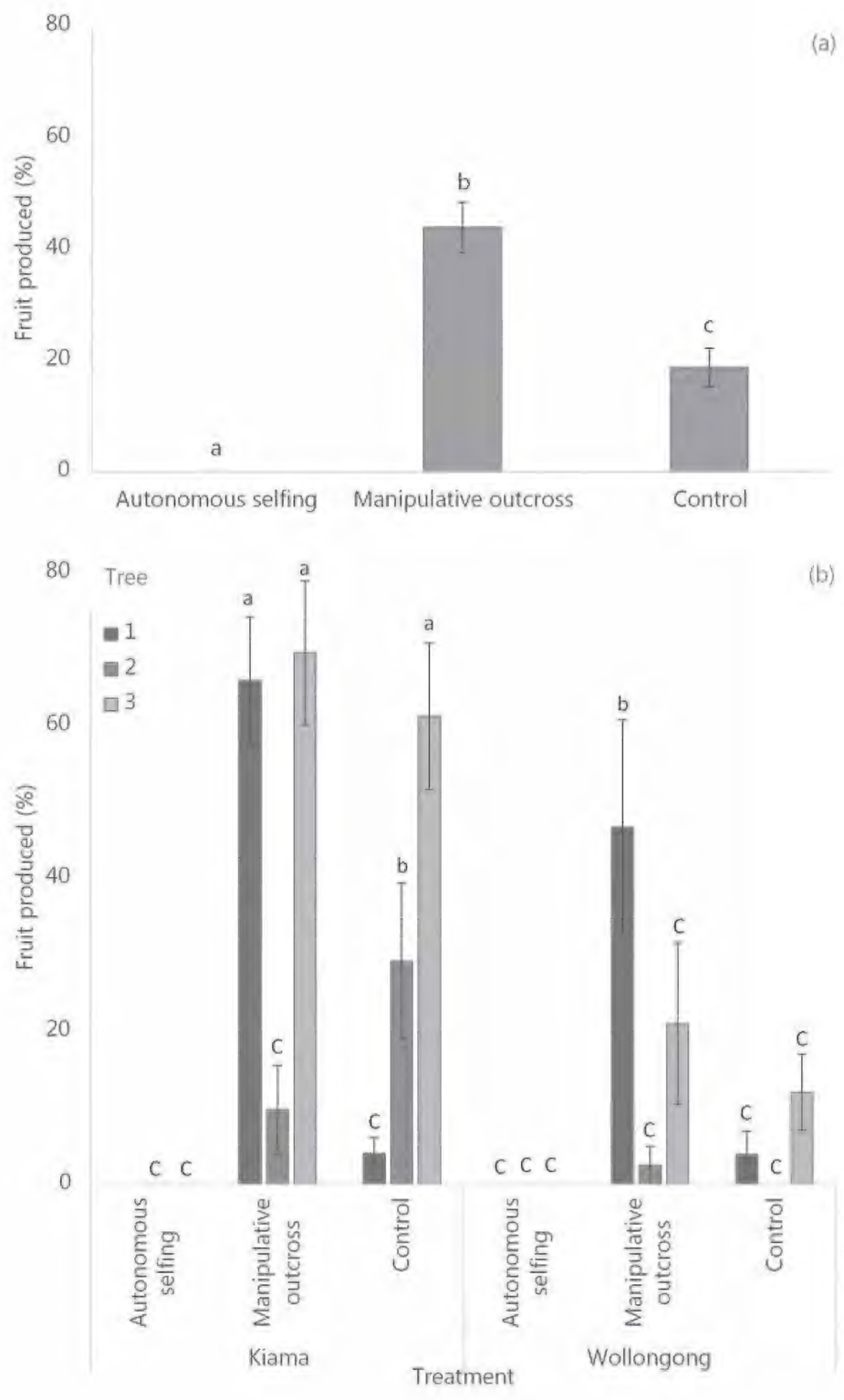

Figure 6. Mean percent (±SE) of *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers that produced fruit following pollination treatments: (a) all treatments on all eight plants (outcross n=89; autonomous selfing n=73; control n=89) and (b) a subset of data where three plants at two site were analysed in a nested three-way ANOVA (n=10). For each panel, bars with different letters are significantly different, according to Tukey HSD analysis after ANOVA.

The seed dissection results indicated that seed viability was variable between sites and treatments; however, 45% of seeds were filled and thus viable (n=810) (Table 2). Merely 16% of seeds collected were empty, while 39% of seeds were partially filled and potentially capable of germination. Insect predation was confirmed within seed collected from Kiama,

South Kiama and Wollongong; but overall, only 3% of seeds collected were predated (Table 2).

Table 1. Analysis of fruit production on each of three *Sarcomelicope simplicifolia* subsp. *simplicifolia* plants at two sites (Wollongong and Kiama). Plant and site were considered random factors and plant was nested within site. Treatment was a fixed factor. Data were arcsine transformed prior to analysis. [*] Depicts significant interaction.

Source	d.f.	d.f. for F	MS	F	P
Site: S	1	1, P(S)	7.905	11.523	0.027*
Plant(site): P(S)	4	4, E	0.686	14.596	< .00001*
Treatment: TM	2	2, P(S) x TM	2.203	3.448	0.083214
S x TM	1	1, P(S) x TM	2.203	3.448	0.083214
P(S) x TM	8	8, E	0.639	13.596	< .00001*
Error: E	161		0.047		

Table 2. Viability of seeds of *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* produced during the manipulative pollination experiments. The number of fruits harvested, and the number of seeds, from each treatment at each site is detailed. The proportion of seeds that were filled, partially filled or empty is provided. The proportion of seeds from each treatment that had signs of insect predation is detailed. The total number of seeds in each seed class (filled, partial filled, empty) is presented for each experimental treatment. The total number of predated seeds is presented as a percentage of the total number of seeds examined below the respective treatment. Shrubs were located at The Australian Botanic Gardens Mount Annan [Mount Annan], Jerrara Dam Reserve [Jerrara], Spring Creek Wetland South [South Kiama], Spring Creek Wetland Kiama [Kiama], and Wollongong Botanic Gardens [Wollongong]. [–] indicates no data obtained.

Treatment	Site	No. fruit	No. seeds	% Filled	% Partial filled	% Empty	% Predated
Acronychia oblongifolia							
Outcross	Jerrara	12	39	41	13	46	0
	Mount Annan	0	0	-	-	-	-
	Total			41	13	46	0
Autonomous selfing	Jerrara	3	4	0	100	0	0
	Mount Annan	4	15	93	0	7	7
	Total			47	50	4	4
No treatment	Jerrara	1	1	0	0	100	0
	Mount Annan	4	32	94	3	3	0
	Total			47	2	51	0
Sarcomelicope simplicifolia* subsp. *simplicifolia							
Outcross	Jerrara	2	3	0	100	0	0
	Kiama	38	305	70	12	18	5
	South Kiama	28	157	9	70	21	1
	Wollongong	33	79	52	27	22	0
	Total			33	52	15	2
Open control	Jerrara	0	0	0	-	-	-
	Kiama	28	172	60	14	26	3
	South Kiama	19	32	3	56	41	9
	Wollongong	0	0	0	-	-	-
	Total			32	35	34	6
Procedural control	Jerrara	1	3	33	66	1	0
	Kiama	15	58	78	9	14	10
	South Kiama	0	0	0	-	-	-
	Wollongong	1	1	100	0	0	0
	Total			70	25	5	3

Discussion

Floral biology

The floral biology of the study species indicated that both fit best into the general entomophilous pollination syndrome. Initial observations of floral phenology found *Acronychia oblongifolia* to be protandrous and *Sarcomelicope simplicifolia* subsp. *simplicifolia* to be dioecious. Protandrous species are typically self-incompatible, while dioecious species are self-incompatible by definition, indicating that both plants likely require a pollen vector for seed set (Bertin & Newman 1993). The highly ornamented pollen walls of both species indicated that they are unlikely to utilise an abiotic pollination vector (Halbritter *et al.* 2018); the heterobrochate reticulum on the exine walls, in particular, is a key indicator of insect pollination (Walker 1976; Sannier 2009) and, specifically, of pollination by beetles and bees (Faegri & van der Pijl 1979). Nectar, the primary floral reward for many pollinators (Kearns & Inouye 1993), was produced by both species, providing further evidence for biotic pollination (Dafni 1992), while the small, white flowers indicated that these two plants are most likely pollinated by a range of insect species (Faegri and van der Pijl 1979).

Pollination vectors

Pollinator assemblages for *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* were rich across all study sites indicating pollinator scarcity is unlikely to inhibit viable seed production in these species. Diptera (flies) were dominant pollinators of both species; insects in this group are known to be significant pollinators of rainforest plants, both in Australia (Williams & Adam 1994) and globally (Barth 1991; Vázquez & Simberloff 2002; Smith-Ramírez *et al.* 2005). This is likely a result of their global distribution and morphological traits (e.g., numerous setae and large bodies) enabling a large pollen carrying capacity (Cook *et al.* 2020). Hymenoptera (bees and wasps) were also frequently detected visiting *Sarcomelicope simplicifolia* subsp. *simplicifolia* and thus, are most likely common pollinators of this species.

A suite of floral visitors identified in our study could be mistaken for pollinators if floral visitation was used as the metric for potential pollinators. Historically, some studies on pollination vectors of Rutaceae have only undertaken observations of floral visitors and not quantified their pollen load (e.g., Armstrong 2002; Sgolastra *et al.* 2016; Pradhan & Devy 2019). Less than one-third of the floral visitors we identified were classed as pollinators, based on foraging behaviour or pollen load analyses (*Acronychia oblongifolia*: 23%; *Sarcomelicope simplicifolia* subsp. *simplicifolia*: 29%). King *et al.* (2013) demonstrated the importance of examining pollen deposition on the stigma and quantifying pollen load to distinguish a floral visitor from a pollinator. However, the same species were rarely detected by both DVR and human observation in our study, preventing validation of potential pollinators using the combined analysis. Future studies should aim to quantify the pollen load of visitors and use a combined analysis of pollen load and pollen deposition on the stigma to confirm whether the species classed as likely pollinators are true pollinators.

Given that no pollen was transported in the *Acronychia oblongifolia* wind pollination experiment, it can be concluded that wind is not a likely pollination vector for this species. Field observations showed pollen of this species to be generally sticky, and SEM observations of the pollen found the exine wall to contain heterobrochate structures, traits that are not typical of species relying on an abiotic pollination vector (Dafni 1992; Walker 1976). This result supports the common view that rainforest plants are not wind pollinated (Bawa & Crisp 1980; Williams & Adam 1994). However, as pollen was dislodged in the *Sarcomelicope simplicifolia* subsp. *simplicifolia* simulations, wind may be a vector, particularly if male and female trees are closely situated. Storms and strong wind gusts would be needed to transport pollen to a pistillate tree, and pollination would be unlikely unless the staminate and pistillate trees were in close proximity. In the populations examined, *Sarcomelicope simplicifolia* subsp. *simplicifolia* trees were adjacent at only two sites, but the canopies never overlapped. This demonstrates wind pollination is possible, but a rare event in the populations studied.

Breeding systems

The results from the breeding system experiment for both species highlight the necessity of biotic pollinators for seed set. For both species, the small percentage of fruit produced from flowers exposed to natural pollinators, compared with those exposed to manually transferred pollen from a different tree, suggests pollen limitation has inhibited fruit production in the populations examined. In rainforests, pollen limitation may result from temporal variation in pollinator abundance and behaviour (Freeman *et al.* 1980; Williams & Adam 2010; Vamosi *et al.* 2013). Extreme weather events, including drought, are known to influence insect behaviour and abundance within rainforest communities (Itioka & Yamauti 2004; Gutiérrez-Fonseca *et al.* 2020). The Sydney Basin Bioregion was classed as being in a drought recovery state throughout the study (DPIE 2020), following the 2019-2020 extreme drought event. To account for the potential effects of this drought on pollinator abundance and behaviour, the reproductive success of both Rutaceae should be examined over an extended period that incorporates multiple seasons.

There is evidence of rainforest Rutaceae producing malfunctioning pollen grains (Armstrong 2002), and pollen viability in some rainforest Rutaceae is known to be low (Auld 2001). The production of non-viable pollen grains could be a contributing factor to the small percentage of flowers exposed to natural pollinators that fruited for both species. Malformed pollen grains were not detected during analysis of pollen ultrastructure; however, no assessment of pollen viability was made. Given the abundance and diversity of pollinators observed visiting these species, short-lived pollen may be one explanation for the poor fruit set. Staining pollen of varying ages would help to determine how long the pollen of these species remains viable (Kearns

& Inouye 1993), and whether short-lived pollen is a factor limiting reproductive success.

Fruit production for both study species varied among trees and sites for some treatments; for example, fruit production from flowers that received experimental outcross pollen varied significantly among trees for *Acronychia oblongifolia*. This spatial variation may indicate that environmental factors have influenced pollination processes and fruit development in these populations. Environmental factors, such as low nutrient availability, can directly reduce fruit production and therefore the reproductive success of rainforest plants (Zagt 1997; Teixeira *et al.* 2006). In a similar study conducted by Adams and Williams (2001), *Acronychia imperforata* F.Muell. failed to develop fruit in any treatment and it was concluded that fruiting success in this species may vary as a consequence of fluctuating resource availability. A greenhouse-based experiment comparing viable seed production after plants are exposed to environmental stress (such as low water or nutrient availability) would aid in understanding the degree to which resource limitation is impacting fruit production.

The level of genetic diversity within each of the populations used in this study may also have influenced fruit production. Jerrara is a restored rainforest community, with *Acronychia oblongifolia* individuals propagated from cuttings from a neighbouring population (D. Black pers. comm.). Therefore, it is possible that the genotypic diversity of some of the *Acronychia oblongifolia* populations examined is limited, thus inhibiting seed production. Similarly, for *Sarcomelicope simplicifolia* subsp. *simplicifolia*, fruit production was significantly less at Wollongong compared with Jerrara. The population at Wollongong, which was situated within a Botanic Garden, consisted of cultivated plants and there is potential that low genetic diversity contributed to poor seed set.

Poor gene flow is one hypothesis for the factors preventing viable seed production in rainforest Rutaceae (Martyn *et al.* 2009). Within the Sydney Basin Bioregion, rainforest habitat is restricted to small pockets now fragmented by clearing for European agriculture (Mills & Jackeman 1995). This fragmentation has potentially increased the distance between conspecific individuals, and in turn, decreased the chance of viable pollen transfer between sexually compatible individuals, which can reduce fecundity in obligate outcrossing species. This reduced fitness can lead to reduced diversity in the population and in turn, inbreeding depression. Both *Acronychia* and *Sarcomelicope* in the Illawarra are both well within their species latitudinal limits, nor are there any major distributional disjunctions evident at the continental scale (Australasian Virtual Herbarium). A genetic analysis of the populations studied would confirm whether inbreeding is impeding viable seed production for *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*.

Finally, evidence of seed predation was apparent for both *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* at several populations, indicating pre-dispersal seed predation is another factor contributing to non-viable seed production in these species. No fruit harvested had a predator present; but a *Megastigmus* (Ichneumonidae) wasp was reared from a single *Zieria granulata* (Rutaceae) fruit from Jerrara, in a study conducted in parallel to the present one (Lopresti *et al*. Under Review). The size and shape of the exit hole in the *Zieria granulata* seed was comparable to the predated *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* seed. There is potential, then, that Ichneumonid wasps are the seed predators for the Rutaceae in this study, but confirmation of the predatory species is required. Importantly, this result indicates that bagging developing fruit to exclude predators may help to improve seed yield for collection.

It should be noted that rainforest habitat within the Sydney Basin Bioregion was restricted before European settlement (Mills 1988) and as a result, low seed production may be a natural state for these plants. Variable seed set may not be an issue for longer-lived trees, provided at least one instance of successful reproduction and establishment occurs within each individual's lifespan. For relatively short-lived trees like *Acronychia oblongifolia* (Floyd 1990), and for tree species suffering reduced life-span due to pressure from climate change (such as with some Australian tropical rainforest Rutaceae, see Bauman *et al.* 2022), this window of opportunity may not be wide enough to sustain the population. This is of particular relevance if land clearing and habitat fragmentation persist, further increasing the distance between conspecific individuals.

Conclusion

This study highlights the importance of insect pollinators for viable seed set in two rainforest Rutaceae species: *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*. Pollen limitation and seed predation was shown to inhibit viable seed production for both species, but this was not the sole limitation. Spatial variation in fruit production indicates that additional factors such as population size, abiotic conditions or poor gene flow among populations are also contributing to non-viable seed production in both *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*. This study is the first to combine an investigation of the floral biology, pollination vectors and breeding system to examine the factors contributing to viable seed production for these species.

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Appendix - Supplementary Material

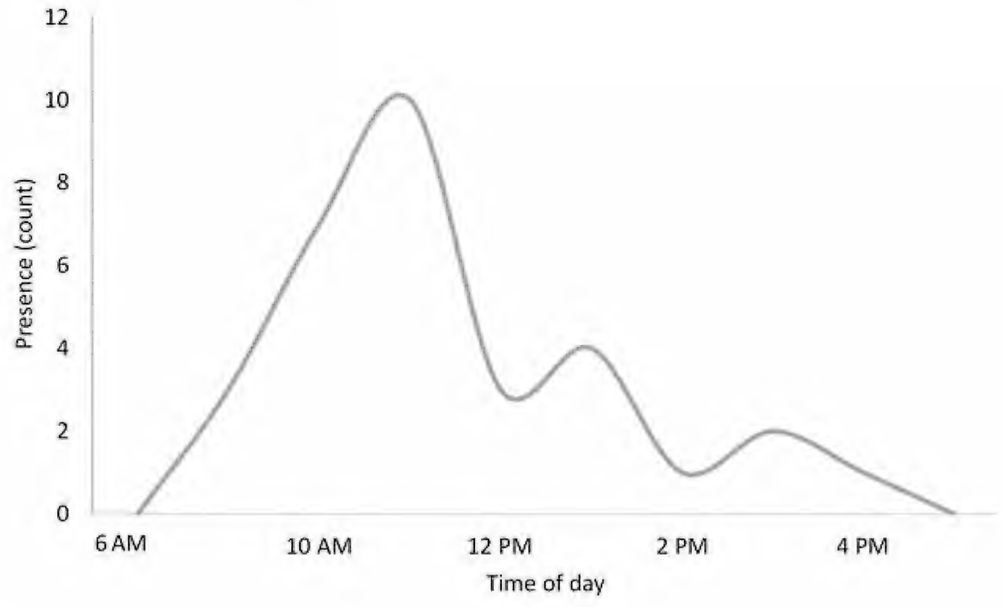

Figure S1. Count of floral visitors foraging on *Acronychia oblongifolia* flowers throughout the day, detected on time-lapse photography cameras recording between sunrise and sunset. Three cameras were set to record on three non-consecutive days at Jerrara (n=108 h).

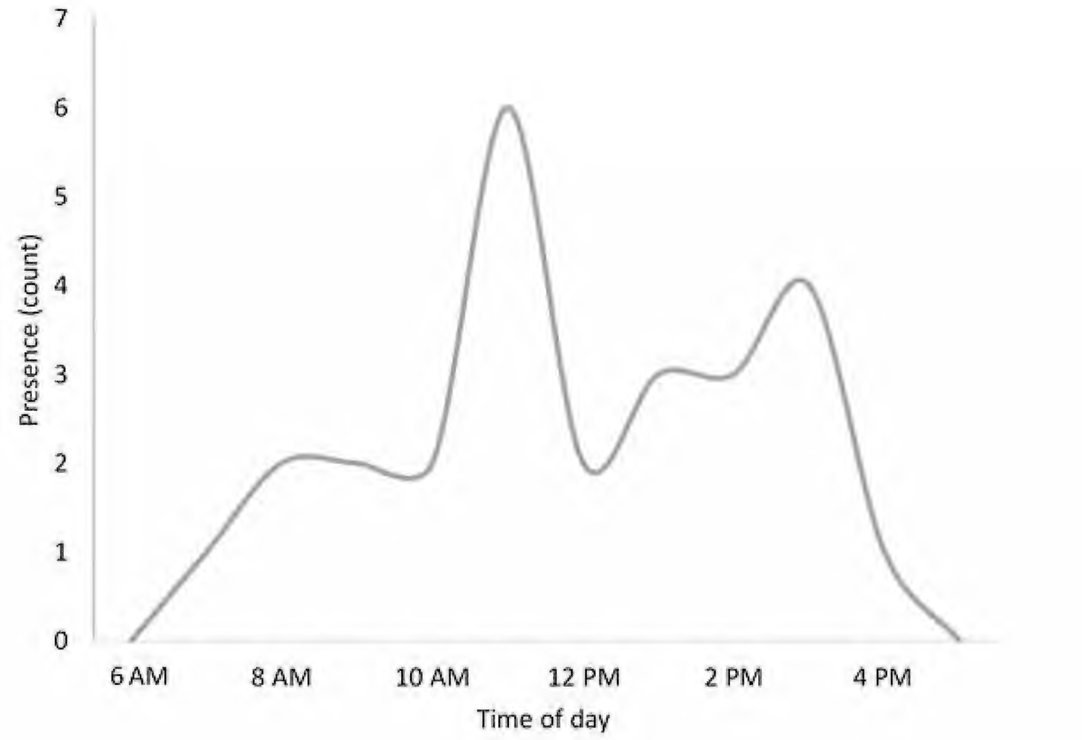

Figure S2. Count of floral visitors foraging on *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers throughout the day, detected on time-lapse photography cameras. Data is pooled across each of four sites, where at each site three cameras were set to record between sunrise and sunset on the same day (n=144 h).

Table S1. Survey effort: total time spent observing floral visitors at each study site and the number of trees observed for each study species, using each of four methods: digital video recordings [DVR], time-lapse photography [TLP], human observations (diurnal and nocturnal) [HO] and nocturnal infrared camera recordings [ICR].

Study Species	Site	No. trees	DVR (h)	TLP (h)	HO (diurnal) (h)	HO (nocturnal) (h)	ICR (nocturnal) (h)
Acronychia oblongifolia	Jerrara	4	30	0	6	9	108
	Mount Annan	2	30	108	6	0	108
Sarcomelicope simplicifolia subsp. *simplicifolia*	Jerrara	2	14	36	6	3	48
	Kiama	4	14	36	6	3	48
	South Kiama	2	14	36	6	3	48
	Wollongong	6	14	36	6	0	48

Table S2. Insects observed contacting flowers of *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* at each study site. Insects were detected by human observation [Direct] or digital video recordings [DVR] or both methods [Both]. Insects were opportunistically captured following their foraging bout and found to carry pollen of the target species [Yes] or not [No]. The percentage of individuals of each species that contacted the stigma while foraging (for species detected on DVR) is detailed. The stigma was not visible in all recordings and these were excluded from this analysis. 'Stigma contact' is the percentage of flowers contacted where the stigma was visible for each species (n is the number of individuals observed). Species that contacted the stigma or carried pollen of the target plant species were considered potential pollinators [*]. [-] Indicates no data. The number of study sites where each species was observed is detailed. Note *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* datasets are independent and unidentified floral visitors observed on each plant species are unique (e.g. "Diptera species 4" observed on *Acronychia oblongifolia* is a different species to "Diptera species 4" observed on *Sarcomelicope simplicifolia* subsp. *simplicifolia*).

Class	Family	Taxon	Insect	Site	Method detected	No. detected on DVR (stigma present)	No. individuals captured	Carrying Pollen	Stigma contact (%) (n)
Acronychia oblongifolia									
Coleoptera	Cantharidae	*Chauliognathus* sp.*	Beetle	1	Direct	0	3	Yes	-
Coleoptera	Chrysomelidae	Luperini sp.*	Beetle	1	Both	3(2)	1	No	100(2)
Coleoptera	Elmidae	Species 1*	Beetle	1	Direct	0	1	Yes	-
Coleoptera	Lycidae	*Trichalus* sp.	Beetle	1	Direct	0	1	No	-

Class	**Family**	**Taxon**	**Insect**	**Site**	**Method detected**	**No. detected on DVR (stigma present)**	**No. individuals captured**	**Carrying Pollen**	**Stigma contact (%) (n)**
Diptera	Calliphoridae	*Chrysomya rufifacies* (Macquart, 1843) *	Fly	1	Both	0	1	Yes	-
Diptera	Calliphoridae	Species 2*	Fly	2	Both	3(3)	2	Yes	100(3)
Diptera	Muscidae	*Dichaetomyia* sp.*	Fly	2	Both	8(7)	4	Yes	100(7)
Diptera	Syrphidae	*Melangyna* sp.*	Hover fly	2	DVR	4(4)	0	-	100(4)
Diptera	Unknown	Species 3	Fly	1	Direct	0	1	-	-
Diptera	Unknown	Species 4	Fly	1	Direct	0	5	No	-
Diptera	Unknown	Species 5	Fly	1	Direct	0	1	No	-
Diptera	Unknown	Species 6	Fly	1	Direct	0	1	No	-
Diptera	Unknown	Species 7	Fly	1	Direct	0	1	No	-
Diptera	Unknown	Species 8-18	Fly	1	Direct	0	1	No	-
Diptera	Platystomatidae	*Pogonortalis* sp.*	Fly	1	Both	15(4)	1	No	100(4)
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758	Honey bee	1	Direct	0	0	-	-
Hymenoptera	Colletidae	*Hylaeus* sp.	Bee	1	Direct	0	1	No	-
Hymenoptera	Formicidae	Dolichoderinae sp.	Ant	1	Both	67(51)	19	No	0.08(51)
Lepidoptera	Erebidae	*Amata* sp.	Tiger moth	1	Direct	0	2	No	-
Lepidoptera	Nymphalidae	*Heteronympha mirifica* (Butler, 1866)*	Butterfly	1	DVR	1(1)	0	-	100(1)
Lepidoptera	Unknown	Species 19*	Moth	1	DVR	1(0)	0	-	0
Sarcomelicope simplicifolia* subsp. *simplicifolia									
Arachnid	Thomisidae	*Sidymella* sp.	Spider	1	Direct	0	4	No	-
Coleoptera	Chrysomelidae	Species 1*	Beetle	1	Direct	0	1	Yes	-
Coleoptera	Coccinellidae	Species 2	Lady beetle	1	Direct	0	1	No	-
Diptera	Calliphoridae	*Calliphora dubia* (Norris 1959)	Fly	1	Direct	0	1	No	-
Diptera	Calliphoridae	*Calliphora* sp. 2*	Fly	3	Both	29(9)	32	Yes	90(9)
Diptera	Calliphoridae	*Calliphora* sp. 3*	Fly	2	Both	1(1)	1	No	100(1)
Diptera	Calliphoridae	*Calliphora* sp. 4	Fly	1	DVR	1(0)	0	-	-
Diptera	Cecidomyiidae	Species 3	Fly	1	Direct	0	1	No	-
Diptera	Chloropidae	Species 4	Fly	2	Both	1(0)	7	No	-
Diptera	Chloropidae	Species 5	Fly	1	Direct	0	2	No	-
Diptera	Chloropidae	Species 6	Fly	1	Direct	0	2	No	-
Diptera	Chloropidae	Species 7	Fly	2	Direct	0	2	No	-
Diptera	Culicidae	Species 8	Mosquito	1	DVR	1(1)	0	-	0(1)
Diptera	Muscidae	*Dichaetomyia* sp.*	Fly	1	Direct	0	5	Yes	-
Diptera	Muscidae	Species 9*	Fly	1	Direct	0	1	Yes	-
Diptera	Muscidae	Species 10	Fly	1	Direct	0	1	No	-
Diptera	Muscidae	Species 11	Fly	1	Direct	0	1	No	-
Diptera	Muscidae	Species 12	Fly	1	Direct	0	1	No	-
Diptera	Platystomatidae	Species 13	Fly	2	Direct	0	2	No	-
Diptera	Sciaridae	Species 14	Fly	1	Direct	0	1	No	-

Class	Family	Taxon	Insect	Site	Method detected	No. detected on DVR (stigma present)	No. individuals captured	Carrying Pollen	Stigma contact (%) (n)
Diptera	Sciaridae	Species 15	Fly	1	Direct	0	1	No	-
Diptera	Syrphidae	*Melangyna sp.**	Hover fly	3	Both	15(0)	6	No	-
Diptera	Tachinidae	Species 16	Fly	2	Direct	0	6	No	-
Diptera	Tachinidae	Species 17	Fly	1	Both	2(0)	2	No	-
Diptera	Tachinidae	Species 18	Fly	2	Direct	0	7	No	-
Diptera	Tephritidae	Species 19*	Fly	1	Direct	0	1	Yes	-
Diptera	Tachinidae	Species 20	Fly	1	Direct	0	1	No	-
Diptera	Unknown	Species 21-30	Fly	3	Direct	0	10	No	-
Diptera	Unknown	Species 31*	Fly	1	Both	2(2)	1	No	100(2)
Diptera	Unknown	Species 32	Fly	1	Direct	3(0)	1	No	-
Hemiptera	Unknown	Species 33	Bug	1	Direct	0	1	No	-
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758*	Honey bee	3	Both	9(4)	10	Yes	100(4)
Hymenoptera	Apidae	*Exoneura* sp.*	Bee	1	Direct	0	3	Yes	-
Hymenoptera	Colletidae	*Hylaeus* sp.*	Bee	1	Direct	0	2	Yes	-
Hymenoptera	Formicidae	Dolichoderinae sp.*	Ant	1	Direct	0	7	Yes	-
Hymenoptera	Formicidae	Species 34	Flying ant	1	Direct	1(0)	1	No	-
Hymenoptera	Halictidae	*Halictus* sp.	Bee	1	Direct	0	1	No	-
Hymenoptera	Halictidae	*Seladonia* sp.	Bee	1	Direct	0	1	No	-
Hymenoptera	Unknown	Species 35	Bee	1	Direct	0	1	No	-
Hymenoptera	Unknown	Species 36	Bee	1	Direct	0	1	No	-

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Horticultural distribution and subsequent naturalization of Queen Palms (*Syagrus romanzoffiana*) in south-eastern Australia

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Abstract: Introduced to Australia in the 1860s, Queen Palm (*Syagrus romanzoffiana*) (family Arecaceae) became a popular ornamental plant in indoor and outdoor settings in the late 19th Century. Queen Palms saw a revival in popularity being heavily promoted in the 1960s and 1970s, resulting in extensive plantings commonly associated with new housing developments and business premises. The propensity of the species to fruit liberally, and its potential in being dispersed by the Grey-headed Flying Fox (*Pteropus poliocephalus*), has led to the plant being declared an environmental weed in some parts of New South Wales and Queensland.

Drawing on data derived from a geographically structured image survey of real estate listings, this paper presents the first distribution map of horticulturally planted and maintained Queen Palms in south-eastern Australia. A comparison with the distribution of naturalized Queen Palms permits us to characterize the biogeographical parameters that circumscribe the horticultural limits of the plant in Australia and the parameters that define its naturalized range.

Keywords: naturalization; weed potential; palms; biogeography; Google™ methodology

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Introduction

Queen Palms (*Syagrus romanzoffiana*) (synonyms *Cocos plumosa, Arecastrum romanzoffianum*), also known as Cocos palm, (family Arecaceae) are plants with pinnate leaves, native to the Atlantic and semideciduous forests of Brazil, Paraguay, Uruguay, and Argentina (Noblick, 2017, p. 180ff). They entered the horticultural trade soon after their discovery as plants of interest (for European eyes) in 1815 with a formal description in 1822 (Choris, 1822, pl. 5–6 and text). Queen Palm are regarded as one of the hardiest of the tropical-looking palms and are suitable to be planted in the USDA plant hardiness 9B (Broschat, 2013) (= Australian zone 3, Dawson, 1991). From the early 1860s onwards, Queen Palms became a popular ornamental plant in Australian outdoor settings, first in Queensland (Hill, 1868), and then in New South Wales (Anonymous, 1870) and Victoria (Anonymous, 1880). The propensity of the species to fruit liberally, creating 'messy' lawns and garden beds (Hortulanus, 1907), saw the popularity of the palm wane in the early 1900s and be eclipsed by the Canary Islands Date Palm (*Phoenix canariensis*) (Spennemann, 2018a, 2018b, 2019). Queen Palm saw a revival in popularity being heavily promoted in the 1960s and 1970s (Anderson, 1964; Seale, 1970, 1974, 1977), resulting in extensive plantings commonly associated with new housing developments and business premises until the 1990s.

Queen Palms had been identified as naturalized and possible sleeper weeds in Australia as early as 1990 (Randall, 2001; (Stanley & Ross, 1989, p. 270). Since then, evidence of naturalization of Queen Palm has been documented in Australasia for tropical and south-eastern Queensland (Batianoff & Butler, 2002; Stephens, 2011; Werren, 2003a), for north-eastern NSW (NC LLS, 2021), as well as on the North Island of New Zealand (Cameron, Sullivan, & Whaley, 2002). At present, there are no confirmed records of naturalisation in South Australia or Western Australia (the record in ALA is a horticulturally planted specimen).

Their dispersal into remnant bushland is facilitated by "flying foxes and other animals" (NSW DPI, 2020) with seeds reputedly readily germinating in riparian areas (Werren, 2003b) and even in dry eucalypt forests (DAF, 2020). Even though classed as a low priority weed (Downey, Scanlon, & Hosking, 2010), some local government areas in south-eastern Queensland and north-eastern NSW have formally identified *S. romanzoffiana* as a weed in their communities (Ballina Shire, s.d.; BCC, 2020; DAF, 2020; NSW DPI, 2020; Gold Coast, n.d.; Logan City Council, 2020; RIC 2019), and/or as a weed of concern (e.g. GS LLS, 2021; NC LLS, 2021). With only two exceptions, the first historical and commencing in 2014 (NSW DPI 2014) but later repealed by the NSW *Biosecurity Act 2015* (a Class 3 [suppression] declaration on Lord Howe Island), and a current local declaration around Bundaberg (Bundaberg Regional Council 2020), local governments have not formally declared the species to be controlled.

A recent observation of Queen Palm drupes consumed, and its seeds dispersed by a Grey-headed Flying Fox (*Pteropus poliocephalus*) (Spennemann, 2020a, 2020b) prompted an assessment of the vectors responsible for dispersal, as well as a need to establish the distribution of the palm species in south-eastern Australia. The Atlas of Living Australia, which relies on data supplied by herbaria, government-agency driven flora surveys as well as crowdsourcing (via iNaturalist for example) suggests a naturalised distribution mainly found in southern Queensland and along the NSW coast as far south as the Ulladulla area (ALA, 2020) with a concentration of observations in the Sydney Basin (Figure 1). It can be expected that the distribution of the plant in horticulturally managed outdoor settings is much greater than its naturalized range.

The aim of this paper is to examine the correlation between the distribution of horticulturally planted and maintained *S. romanzoffiana* and the distribution of the palm species in their naturalized state.

Methodology

Since, at least in southern New South Wales, Queen Palms are commonly associated with residences and businesses erected in the late 1970s to 1990s (pers. obs.), the distribution of the Queen Palm in managed horticultural settings (public and private gardens, landscaping of commercial properties) was assessed using a rapid image survey of real estate listings and other evidence.

To identify the presence or absence of Queen Palm in a given locality, the rapid survey used the Google™ image search function (https://images.google.com, accessed between 12 May and 12 June 2020). The town names to be searched were selected from inspection of regional maps (using Google-Maps™), with an emphasis on a broad regional coverage. A preliminary search for a few towns using the keyword combination "name of town + palm" noted a high prevalence of images of residential properties with palms published as part of listings by real estate agents. Thus, the main search logic employed was the keyword combination "name of town + real estate" with the combination "name of town + palm" used as a fall back. If images were located, the associated link was followed, the palm verified as to the desired species, and the respective street address extracted. The coordinates of each observation were determined by entering the street address into GoogleMaps™ and extracted from there. A single record of presence sufficed for locational verification.

Some of the searches brought up imagery of properties with Queen Palm in localities that had not been specifically targeted (for example where a street name contained the name of the community searched). These opportunistic observations were added to the data set.

Where the presence of Queen Palm was not obvious in the initial searches, additional searches were carried out using the keyword combination: "name of town + motel | hotel |

caravan [park].” Experience in initial searches yielded many images of motels (esp. motel pools) and caravan parks with Queen Palms.

Where the presence of Queen Palms could still not be determined but was likely, given the presence of such palms in surrounding communities, Google StreetView™ was used for a virtual drive-through of residential neighbourhoods, with priority given to suburbs established during the 1980s and 1990s (if they could be identified as such from the Satellite View). The data search was carried out between 12 May and 12 June 2020. As far as it is known, the use of location-focussed Google images searches to Google StreetView™ is a methodological first for compiling a plant distribution map.

Results

In total 319 locations were assessed, with 60 locations returning no evidence of Queen Palm. The locations where the occurrence of Queen Palm could not be confirmed are shown as small dots in the distribution map (Figure 2). While the naturalized occurrences of Queen Palm are confined to the eastern seaboard (Figure 1), horticulturally maintained specimens can be found throughout subtropical and warm temperate regions of south-eastern Australia (Figure 2). The observed horticultural distribution far exceeds the area where Queen Palm have been found to be naturalized.

Discussion and Conclusions

The distribution of naturalized *Syagrus romanzoffiana* in south-eastern Australia is confined to a narrow coastal strip from southern Queensland and along the NSW coast as far south as the Narooma area (Figure 1) (The ALA record for Eden is an outlier and may represent a cultivated example). Climatologically, this coincides with areas in SE Australia that exhibit more than 1000 mm annual rainfall (Bureau of Meterology, 2023b) and where mean temperatures do not drop below 12°C (Bureau of Meterology, 2023a).

The species is absent in the arid and semi-arid areas of New South Wales, South Australia and Queensland. An exception occurs in a suburban garden in Broken Hill, where the water requirements are apparently met artificially. It shows that the Queen Palm can survive in areas which experience less than 200 mm annual rainfall (Bureau of Meterology, 2023b) and that exhibit average summer temperature maxima above 30°C (Bureau of Meterology, 2023a).

The distribution map shows some areas where Queen Palm are absent, even though they are, on a regional scale, surrounded by locations where such palms are present. Some of these absences seem to be true reflections of climatological constraints (e.g. Southern Tablelands, Australian Alps or Tasmania). The data show that *S. romanzoffiana* is cold-intolerant and is absent in areas where mean temperatures drop to 6°C or below (Bureau of Meterology, 2023a).

Other absences are likely to be false negatives from a biogeographical perspective because the absence cannot be readily explained by climatological constraints and palms are known from horticultural settings in nearby places. This particularly applies to the Southern Volcanic Plain bioregion of southwest Victoria. Given that the search method was the same and given that climatic factors can be ruled out, it can be surmised that the absence is due to socio-economic or cultural factors. The mapping is based on the presence of horticultural plantings at residential properties or commercial premises, which are driven by a property-owner’s personal interest, which in turn is influenced by architectural fashion and landscape design. Thus, the absence of Queen Palm in the smaller rural farming communities in the central part of southwestern Victoria may be an artefact of different community expectations. It may also reflect socio-economic or cultural conditions prevailing in these regions, which saw the palm craze of the 1970s to 1990s pass them by, and suggests a fruitful avenue for further work, exploring the intersection of the planting of ornamental palm species (Queen Palms, Canary Islands Date Palms etc) with the socio-economic and cultural fabric of communities in rural and regional Australia.

Figure 1. Distribution of *Queen palm Syagrus romanzoffiana* in south-eastern Australia: naturalized occurrences. Data: Atlas of Living Australia. (ALA, 2020).

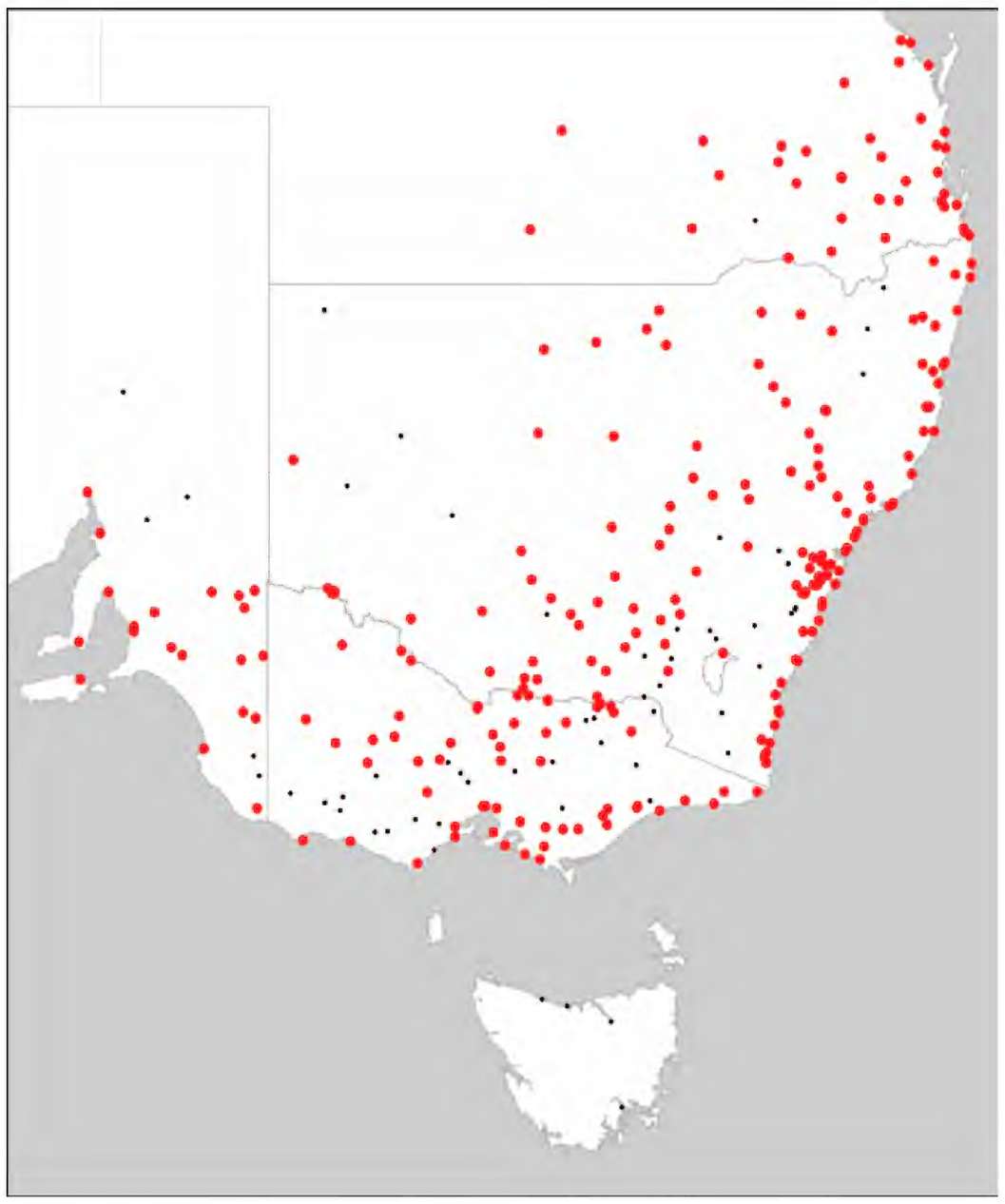

Figure 2. Distribution of Queen Palm *Syagrus romanzoffiana* in south-eastern Australia: horticultural settings. Small dots indicate locations where no *S. romanzoffiana* could be located during the on-line searches.

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Island treasures: rapid assessment of orchids on Norfolk Island

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***Abstract*:** Islands, because of their small geographic area and tendency for high levels of species endemism, present opportunities for comprehensive biodiversity assessment and conservation. Norfolk Island (35 km^2 in area) is in the South Pacific Ocean, between New Zealand and New Caledonia, approximately 1500 km east of Australia. To assess the conservation and taxonomic status of the orchids of Norfolk Island we analysed historical literature and herbarium records, and then conducted a 10-day field survey. We made 91 records of 11 orchid species, including the first record of *Pinalia rostriflora* (Rchb.f.) Kuntze for the island. The orchid flora of Norfolk Island is relatively species rich, compared to other small oceanic islands.

Because of Norfolk Island's small land area, its endemic orchids may be eligible for listing as Critically Endangered under Australian Government legislation (the *Environment Protection and Biodiversity Conservation Act* 1999), due to their restricted geographic range. Targeted assessments, such as this, raise awareness of the presence and distribution of threatened species, and give confidence to land managers in prioritising conservation management actions.

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Introduction

Biodiversity decline is in the spotlight globally (United Nations 2015, 2023, WWF 2022). As resources for biodiversity conservation are limited (Wintle et al. 2019), prioritisation of conservation actions to achieve the maximum benefit has been advocated (Chades et al. 2008, Joseph et al. 2009). Species that are listed as threatened (at state, national or international levels) are typically prioritised for conservation management (Hoffman et al. 2008), but this can be problematic because many species are yet to be assessed (e.g., plants; Le Breton et al. 2019, Alfonzetti et al. 2020). Taxonomic uncertainty adds further complication to prioritisation, introducing ambiguity in relation to target taxa and actions (Eldridge et al. 2014).

Biodiversity conservation efforts are largely focussed on species (Mace et al. 2008). However, with the advent of genomics technologies and increasing awareness of within-species diversity, our understanding of global biodiversity – and biodiversity declines – is deepening (Des Roches et al. 2021, Formenti et al. 2022). Calls are growing for a more nuanced approach to conservation, one that considers not only species, but the processes that lead to speciation, and the protection of genetic diversity at levels below species level (e.g., subspecies, and genetically and/or geographically distinct populations) (Coates et al. 2018, Des Roches et al. 2021).

Islands provide a valuable environment for advancing our understanding of biodiversity (MacArthur and Wilson 1967). Their discrete area makes comprehensive biodiversity assessment more tractable. Islands represent less than 7% of the world's emergent land but hold 20% of its biota (Sayre et al. 2019). In addition, islands host a high percentage of endemic species (Fernandez-Palacios et al. 2021), and often exhibit a high species-to-area ratio, which combined is hypothesised to offer higher returns for conservation effort (Kier et al. 2009). Islands also offer a particular challenge for managing threats to biodiversity (such as invasive species and disease; Russell and Kueffer 2019, Fernandez-Palacios et al. 2021). Because of their discrete areas and many endemic species, options for dispersal or assisted migration to suitable climates/locations are limited (Courchamp et al. 2014).

Orchids (family Orchidaceae) are among the most threatened plants in the world: globally, more than half the orchid taxa assessed for the IUCN Red List were determined to be threatened; however, <5% have been assessed (Fay 2018). Australia harbours a rich and highly endemic orchid flora with more than 90% occurring nowhere else (Jones 2021). Orchids constitute a large proportion of Australia's threatened flora (16%) and 13% of Australia's orchids are nationally listed as threatened (Australian Government 2023, Jones 2021). Most of Australia's orchids are terrestrial (86%) and exist for part of the year as an underground storage organ only (i.e., they are invisible above ground during this period). Hence, detecting orchids in the field can be challenging. Additionally, terrestrial orchids do not necessarily flower annually (Jones 2021, Shefferson 2004). There are also challenges in effectively surveying epiphytic orchids, which constitute 14% of Australia's orchid flora (Jones 2021), as they occur high in the canopies of trees; indeed, one published survey method for epiphytes states frankly: "not all species will be detected" (Shaw and Bergstrom 1997). The Orchidaceae is also one of the largest and most complex plant families, resulting in many areas of taxonomic uncertainty (Fay 2018). The challenges in taxonomic circumscription of orchids are well known (Bateman 2012, Fay 2018), as are the flow on effects to conservation, especially where there are differing species concepts (Frankham et al. 2012).

Extinction risk assessment typically includes three main elements: geographic distribution, population, and rate of decline (IUCN 2022a). Ninety per cent of the 272 orchids listed as threatened on the IUCN Red List in the past 10 years have been assessed under Criterion B (IUCN 2022b), which focuses on geographic distribution, rather than population. Assessment of whether a species is continuing to decline, in distribution or population, is important in extinction risk assessment, as is identifying the most serious plausible threat most likely to rapidly affect the population (IUCN 2022a). For a species to be eligible for listing as Critically Endangered because it has a restricted geographic distribution, it needs to meet two of three IUCN Red List sub-criteria, these are: existing in one threat-defined location or being severely fragmented; undergoing continuing decline; and/or undergoing extreme fluctuations in population.

Orchids are often threatened, and can be taxonomically difficult. Islands have a high proportion of endemic species, small areas compared to continents, and significant barriers to genetic exchange. For these reasons, the study of orchids on islands is likely to yield information important for taxonomy and conservation. Here, we present Norfolk Island as a case study of a rapid assessment of the taxonomic and conservation status of an island orchid community. We use historical and contemporary data to assess the taxonomic and conservation status of the orchid flora of Norfolk Island, at both the species and population level, to assist with prioritisation of conservation resources. We also make recommendations about on-ground management and further research.

Methods

Study site: geography

Norfolk Island is situated in the South Pacific Ocean, approximately 1500 km east of Australia, between New Zealand and New Caledonia, at approximately the same latitude as Evans Head, NSW. The climate is subtropical and oceanic (Green 1994). Norfolk Island covers an area of 34.6 km^2, with volcanic geology. The highest point is the peak of Mt Bates, at 321 m above sea level. Norfolk Island was formed ~2.3 – 3.1 Mya (Jones and McDougall 1973; DNP 2010); it is younger than New Caledonia (~50 Mya) and Lord Howe Island (~7 Mya), but older than Tahiti (0.6-1.2 Mya) (MacDougall et al. 1981, Neall and Trewick 2008).

Study site: history

Archaeological evidence suggests that Norfolk Island was inhabited by Polynesians, but their settlement ended before Europeans arrived (Power 2022). Norfolk Island was settled by Europeans soon after establishment of a convict settlement in Sydney in 1788. At this time the island was almost entirely covered in subtropical rainforest, with *Araucaria heterophylla* a dominant feature (Hicks 1988). The first botanical collections of Norfolk Island plants (not including orchids) were made in 1774, when Captain Cook landed on the island, and these were described by Georg Forster. It was not until botanical illustrator and collector Ferdinand Bauer visited the island in 1804-1805 that a comprehensive botanical study was undertaken. Bauer's work informed the *Prodromus Florae Norfolkicae* by S.F.L. Endlicher (1833) who described five new orchid species (Table 1). In the years following, there were collections by Allan Cunningham (botanist at the Botanic Gardens in Sydney) in 1830 (Mills 2012) and James Backhouse in 1835, although neither appear to have included orchids. By the end of the convict era in the 1850s, the island had been largely cleared for agriculture, and pigs and goats were introduced. Isaac Robinson, a resident of the island, made at least three orchid collections (1884-1885). Gertrude Purchas, who later records indicate was an artist from New Zealand, made the only collection of the orchid *Nematoceras acuminata* in ~1894, and sent the specimen to Thomas Kirk, a New Zealand botanist, who referred it to Ferdinand von Mueller at the Melbourne Botanic Gardens (Mills 2007a). Further orchid records were made by Captain J.D. McCormish in 1937; in 1947 (date estimated based on a related collection [PDD 5580]) by W. Cottier, and; in 1956 by W. Reginald B. Oliver (later Director of the Dominion Museum, NZ). Additional collections have been made more recently (in the past 70 years) by other collectors.

Table 1. Summary of records for orchid species on Norfolk Island. Abbreviations for other localities to which these orchids extend include Lord Howe Island (LHI), New Zealand (NZ), and New Caledonia (NC). * = Date collected as accessioned herbarium specimen.

	Species	Date first collected	Date last collected*	Number of collections	Records limited to NI/endemism (other locations where species is or may be present are in brackets)	Threatened species listing status	Informal assessment of rarity on NI
1	*Adelopetalum argyropus* (Endl.) D.L.Jones & M.A.Clem.	1804 (Bauer) – type	1996	5	No (Mainland Australia, LHI)	Qld: VU	Very rare
2	*Nematoceras acuminata* (M.A.Clem. & Hatch) Molloy, D.L.Jones & M.A.Clem.	1864 (Purchas)	1864	1	No (NZ)	None	Very rare
3	*Microtis parvifolia* R.Br.	1885 (Robinson)	1885	1	Unclear	None	Uncertain
4	*Microtis unifolia* (G.Forst) Rcbhf.	1937	1956	3	No (Australia, Pacific, Asia)	None	Uncertain
5	*Oberonia titania* Lindl.	1804 (Bauer) – type	2018	3	No (Mainland Australia)	NSW: VU	Rare
6	*Phreatia paleata* (Rchb.f.) Rchb.f. ex Kraenzl.	1975	1975	1	No (Pacific)	EPBC: EN	Common
7	*Phreatia limenophylax* (Endl.) Benth.	1804 (Bauer) – type	2018	2	No (also NC – where it is rare)	EPBC: CE	Very rare
8	*Taeniophyllum norfolkianum* D.L.Jones, B.Gray & M.A.Clem.	1967 (Hoogland) – type	1996	3	Yes	EPBC: VU	Uncommon
9	*Thelychiton brachypus* Endl.	1804 (Bauer) – type	?1804	1	Yes	EPBC: EN	Rare
10	*Thelychiton macropus* Endl.	1804 (Bauer) – type	2018	6	Yes	None	Common
11	*Thelymitra longifolia* J.R.Forst. & G.Forst.	1998	1998	1	No (NZ)	None	Uncertain – Rare
12	*Tropidia viridifusca* Kraenzl.	1997	2016	5	No (Pacific)	None	Common

Study site: vegetation

Remnant native vegetation is found across 425 hectares of Norfolk Island, or 12% of the total land area. Most remnant vegetation is located within the Mount Pitt Section of the Norfolk Island National Park (460 ha) on the northern side of the island (Christian and Mills 2021). The vegetation of Norfolk Island is classified into eight forest vegetation types and six non-forest vegetation types (Christian and Mills 2021). Large areas of Norfolk Island are also covered by non-remnant vegetation including woody weed forest and cleared pastures. Important woody weed species include red guava (*Psidium cattleyanum*), Brazilian peppertree/broad-leaved peppertree (*Schinus terebinthifolius*) and African olive (*Olea europaea* subsp. *cuspidata*). These species form dense thickets, have allelopathic effects and dominate resources such as light and water, making it difficult for other species to compete (DNP 2010).

Data review

We began by identifying which orchid species had been previously recorded on Norfolk Island. First, we downloaded all records of "Orchidaceae" on Norfolk Island from the Atlas of Living Australia (ala.org.au; ALA). To capture additional records without (or with incorrect) coordinates, we manually searched all Orchidaceae records containing the word "Norfolk", crosschecking with other collection information (location, collector, date, habitat and occurrence notes). In this way we gathered 26 verifiable records of 10 species.

We then searched Global Plants (https://plants.jstor.org/), a global database of digitised herbarium specimens, for specimens from Orchidaceae containing the word "Norfolk", to identify additional specimens not included in ALA. Once specimens from other localities had been removed, this revealed seven specimens of five taxa, all from Ferdinand Bauer in 1865. Among these was one species additional to those retrieved from the ALA database: *Thelychiton brachypus* Endl.

A review of key literature (publications focused on or with comprehensive listings of Norfolk Island flora i.e., Green 1994; Mills 2007b; Mills 2010; DNP 2010; Backhouse et al. 2019) revealed one additional species: *Thelymitra longifolia* J.R.Forst. & G.Forst. (de Lange et al. 2005). We then used the Species Profiles and Threats database (http://www.environment.gov.au/cgi-bin/sprat/public/sprat.pl; SPRAT) to determine the current threatened species listing status for each of the 12 species (Table 1). For scientific orchid names we use the taxonomy of Jones (2021). Common names of orchids are listed in Appendix 1.

Field survey

Over 10 days in May 2022, we set out to visit the locations recorded for the herbarium specimens, in the literature and known by Norfolk Island National Park staff. We began by visiting areas with the largest number of orchid records. These were largely within Norfolk Island National Park (the Park). The highest concentration of records was on the slopes of Mt Pitt. We then visited Norfolk Island Reserves: Bumboras, Cascade, Cockpit, Crystal Pool, Headstone, Hundred Acres, Selwyn and Two Chimneys, with opportunistic surveys along roadsides during transit. Survey was on foot, using binoculars, with two to eight people in attendance. Orchid locations were recorded using GPS, and voucher specimens were taken where a species was recorded in a new area. Short opportunistic visits (<2 days fieldwork) were also made in October 2022 (M. Clements) and December 2022 (H. Zimmer).

Assessment

Rapid conservation assessment was made based on the IUCN Red List Categories and Criteria (2022a). Using the historical and field survey records, we were able to assess the current conservation status of the orchids of Norfolk Island, largely based on IUCN Criterion B, which has a focus on geographic distribution (i.e., occurrence records with latitude and longitude). Extent of occurrence (EOO) and area of occupancy (AOO) were calculated according to Red List guidelines i.e., EOO as the area of a minimum convex hull encompassing all records, and AOO calculated as the number of 2 x 2 km grid cells occupied by species records (IUCN 2022). EOO and AOO were calculated for Norfolk Island endemic species and distinct populations. We did not explicitly assess IUCN Red Listing subcriteria (i.e., number of locations, continuing decline). We use the term 'Norfolk Island population' for orchid species on Norfolk Island that may be distinct but, as currently circumscribed/delimited, have distributions that extend beyond Norfolk Island (e.g., also present in Australia or New Zealand).

Results

Species list

We detected ten of the 12 species of orchid known from Norfolk Island (Figures 1 and 2) as well as one species not previously recorded from the Norfolk Island Group: *Pinalia rostriflora* (Rchb.f.) Kuntze, Revis. Gen. Pl. 2: 679 (1891).

The two species not seen were terrestrial orchids: *Nematoceras acuminata* and *Thelymitra longifolia*. *Nematoceras acuminata* has been recorded on Norfolk Island only once, by Gertrude Purchas, who gathered a specimen in about 1894 (Mills 2007). The second species not seen, *Thelymitra longifolia* was recorded in 1998 (de Lange *et al.* 2005).

In addition, two species from the genus *Microtis* were identified as occurring on Norfolk Island. *Microtis aemula* was found in May 2022 and *Microtis unifolia* was identified at a separate location on an opportunistic visit to Norfolk Island (by M. Clements) in October 2022.

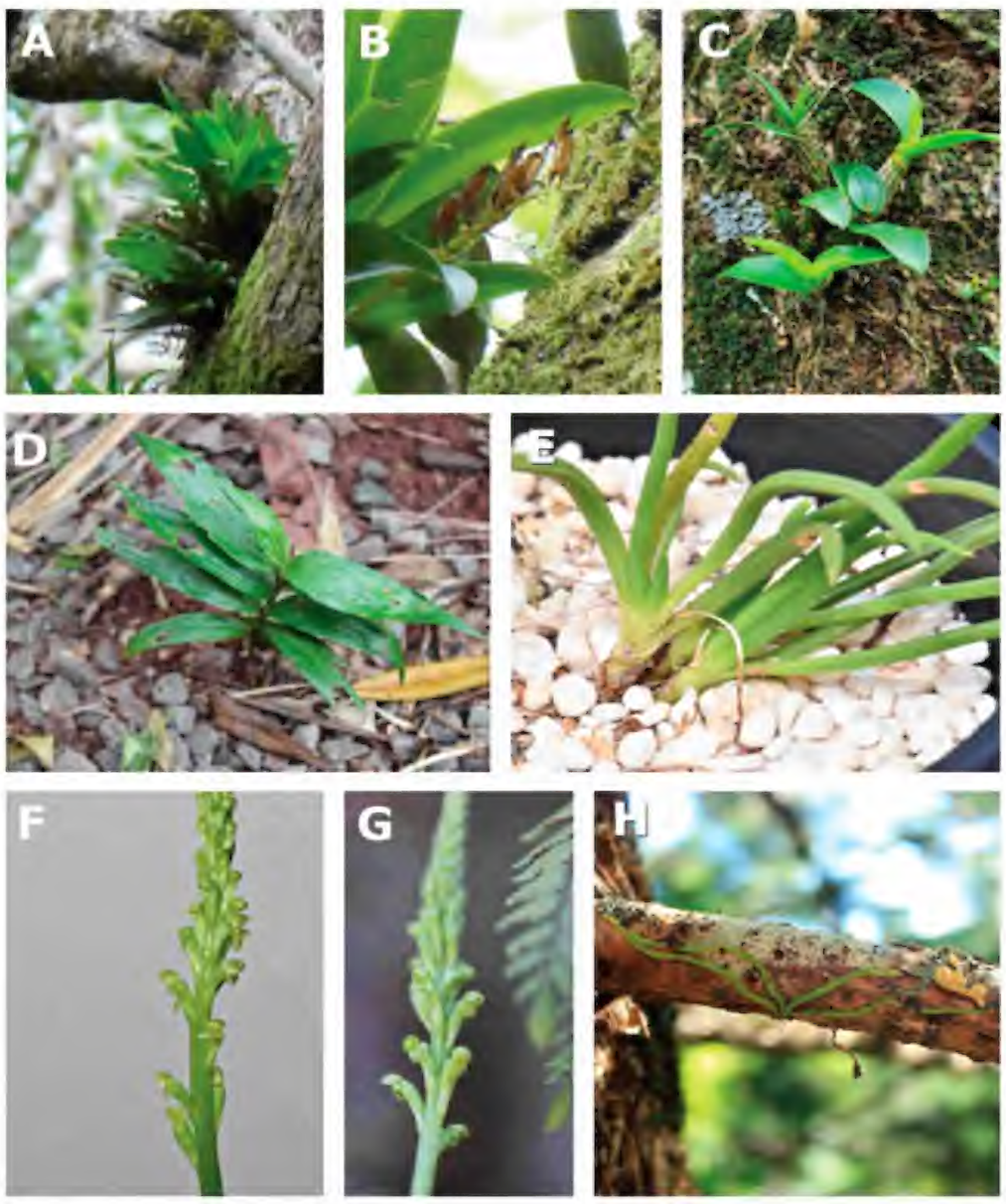

Figure 1. Orchids of Norfolk Island plate 1: (A, B) *Pinalia rostriflora*; (C) *Thelychiton brachypus*; (D) *Tropidia viridifusca*; (E) *Phreatia limenophylax*; (F) *Microtis aemula*; (G) *Microtis unifolia*; (H) *Taeniophyllum norfolkianum*. Photo credits: Mark Clements (A, B, E, F, G), Heidi Zimmer (C, D, H).

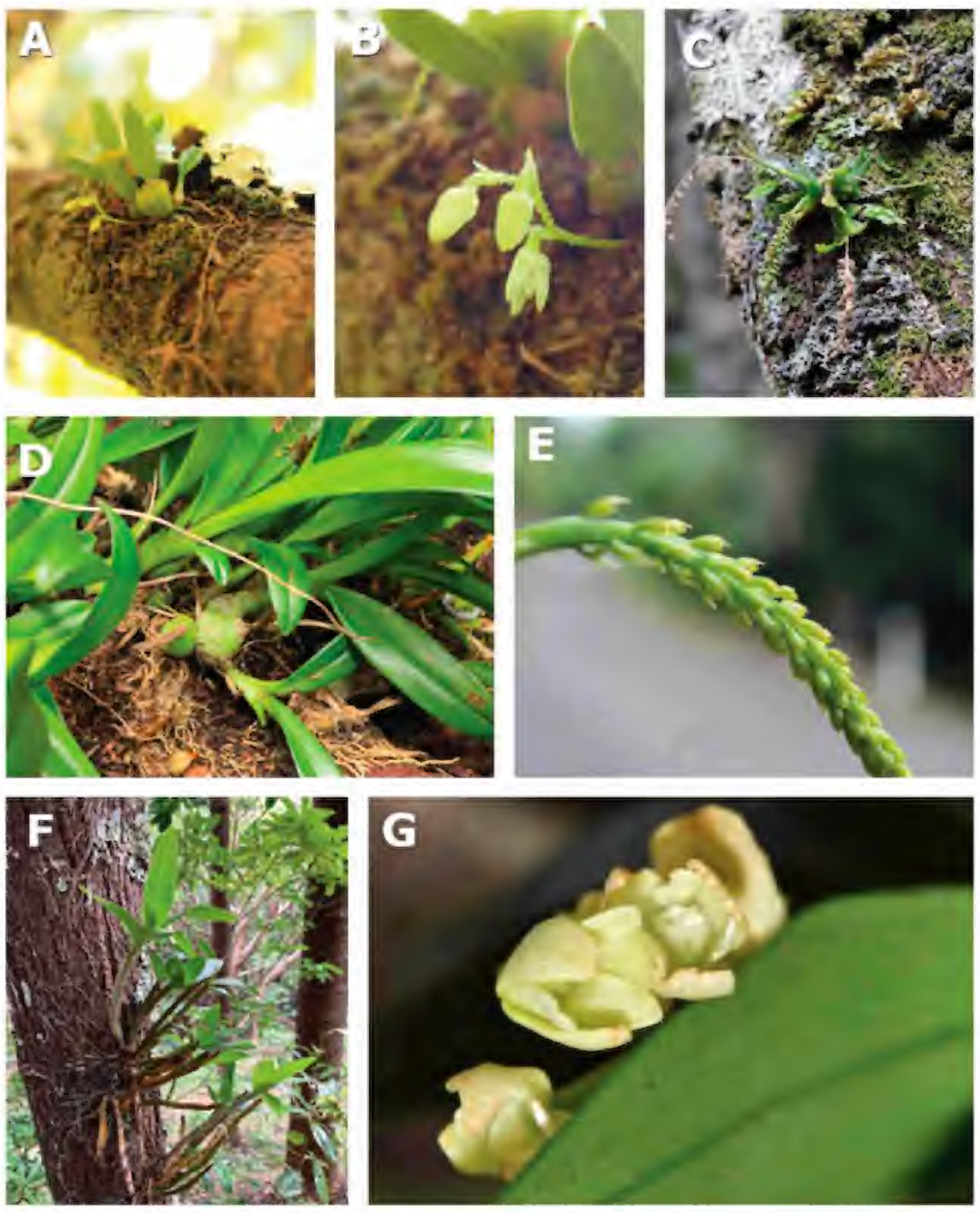

Figure 2. Orchids of Norfolk Island plate 2: (A, B) *Adelopetalum argyropus*; (C) *Oberonia titania;* (D, E) *Phreatia paleata;* (F,G) *Thelychiton macropus*. Photo credits: Mark Clements (B, G), Heidi Zimmer (A, C, D, E, F).

Rapid conservation assessment: distribution

The majority of orchids were recorded within Norfolk Island National Park (Figure 3), consistent with the spatial pattern of previous records. Because of the small geographic area of Norfolk Island, any species (or subspecies or population) endemic to Norfolk Island meets the geographic threshold (EOO <100 km^2) for listing as Critically Endangered (CE) under IUCN Red List Criterion B1. In addition, eight species or populations also met the threshold for listing as Critically Endangered under Criterion B2, having an AOO <10 km^2. Summary data for species and populations restricted to Norfolk Island are given in Table 2. Other thresholds, such as number of locations and decline, must be met, however, before a species can be assessed as CE (IUCN 2022a).

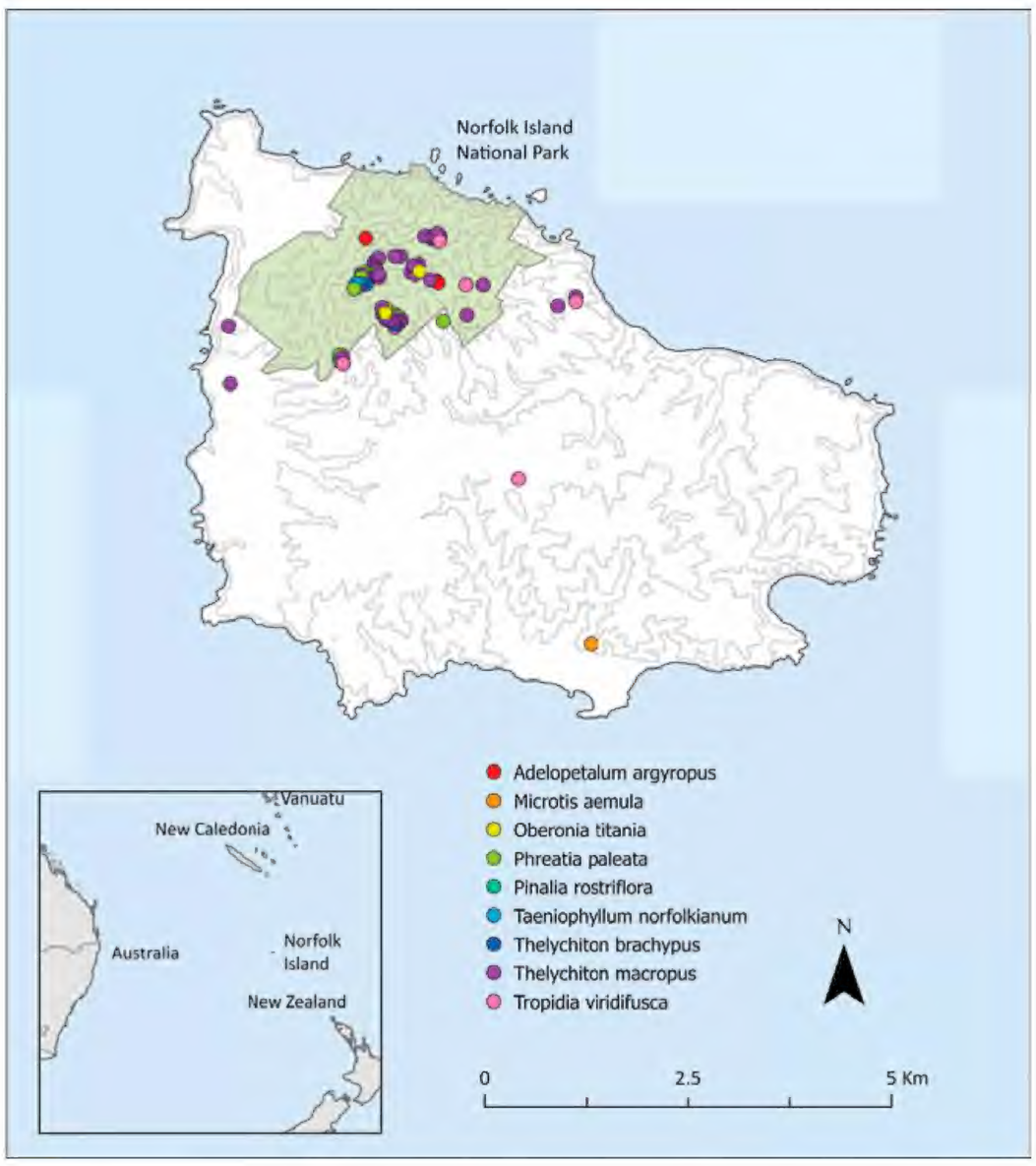

Figure 3. Map of Norfolk Island showing Norfolk Island National Park orchid records. Specific locations of orchid records have been generalised.

Taxonomic assessment

At least one orchid species from Norfolk Island appears to be morphologically distinct from populations of that species on continental Australia. The population of *Adelopetalum argyropus* on Norfolk Island has roughly spherical to inverted-cone-shaped pseudobulbs, which are ribbed or grooved, and flowers that do not open widely, with labellum not protruding. This is distinct from the mainland populations that have egg-shaped to conical pseudobulbs with weakly ribbed and irregularly verrucose surface, and have flowers that open widely, with labellum protruding.

Table 2. Rapid conservation assessment based on geographic range according to the IUCN Red List criteria. Factors considered for listing based on geographic distribution include extent of occurrence (EOO) and area of occupancy (AOO). EOO and AOO were calculated for records on Norfolk Island only. Species for which this does not constitute a global assessment are annotated as 'NI population'. Assessment of continuing decline is based on Norfolk Island occurrences only. Additional abbreviations are: CE, critically endangered; EPBC Act, Environment Protection and Biodiversity Conservation Act (1999); NC, New Caledonia; NZ, New Zealand; NI, Norfolk Island.

Species name	Synonyms	EOO (km^2)	AOO (km^2)	Most important threat causing continuing decline	Conservation assessment (for EPBC Act) recommended	Further taxonomic work recommended (to delimit NI species/populations)
Adelopetalum argyropus NI population	*Bulbophyllum argyropus* NI population	12	12	Restricted habitat and climate change	Yes. New conservation assessment for NI population.	Yes. Delimitation re. continental Australian *Adelopetalum argyropus* populations. Reference phylogenetic framework: Simpson et al. (2022).
Nematoceras acuminata *	*Corybas acuminatus*	NA	NA	Likely extinct	No. Not recorded on NI for >150 years. Species present in NZ.	No
Microtis aemula NI population	none	4	4	Grazing	Further research required.	Yes. Species delimitation in *Microtis.*
Microtis unifolia NI population	none	4	4	Grazing	Further research required.	Yes. Species delimitation in *Microtis.*
Oberonia titania NI population	none	4	4	Restricted habitat and climate change	Further research required.	Yes. Species delimitation in *Oberonia* including Australian populations (conspecifics) and Australian and New Caledonia relatives, especially: *O. crateriformis O. neocaledonica, O. palmicola, O. rimachila.*
Phreatia paleata NI population	none	8	8	Restricted habitat and climate change	Yes. Reassessment. Only Australian occurrence of this species is on NI.	Yes. Compare with conspecifics in the Asia-Pacific.
Phreatia limenophylax NI population	*Pleaxure limenophylax* NI population	4	4	Restricted habitat and climate change	No. Species is listed as CE.	Yes. Compare with conspecifics in New Caledonia.
Pinalia rostriflora NI population	*Eria rostriflora* NI population	4	4	Restricted habitat and climate change	Yes. Only Australian occurrence of this is on NI.	Yes. Compare with conspecifics in New Caledonia and elsewhere in the Pacific.
Taeniophyllum norfolkianum	none	8	8	Restricted habitat and climate change	Yes. Reassessment. The global distribution of this species is on NI.	No
Thelychiton brachypus	*Dendrobium brachypus*	4	4	Restricted habitat and climate change	Yes. Reassessment. The global distribution of this species is on NI.	Delimitation with respect to *Thelychiton macropus*.
Thelychiton macropus	*Dendrobium macropus*	20	20	Restricted habitat and climate change	Yes. The global distribution of this species is on NI	Delimitation with respect to *Thelychiton brachypus.*
Thelymitra longifolia *	none	NA	NA	Grazing	Further research required into species delimitation in *Thelymitra.*	Delimitation with respect to populations of the same species in NZ. Reference phylogenetic framework: Nauheimer et al. (2018).
Tropidia viridifusca NI population	none	16	16	Restricted habitat and climate change	Yes. Only Australian occurrence of this species is on NI.	No

* Species not seen *in situ* for this study (i.e., *Nematoceras acuminatus, Thelymitra longifolia*).

**The type location for this species was recorded as Anson Bay; however, because remnant vegetation has largely been cleared in this area, and we were unable to find any extant *P. limenophylax* here, estimates of EOO and AOO do not include an Anson Bay location.

Spatial correlations among species and vegetation types

Orchids were recorded from four vegetation types on Norfolk Island. The majority of records were moist upland hardwood forest (MUHF) (Figure 4), which has an area of 108 hectares (or 3% of Norfolk Island) (Christian and Mills 2021). MUHF is typically found on the slopes and valleys around the mountains, between the moist palm valley forest and the pine-hardwood ridge forest vegetation types, which cover 42 ha and 163 ha, respectively (Christian and Mills 2021), and are the other two vegetation types where orchids were predominantly recorded.

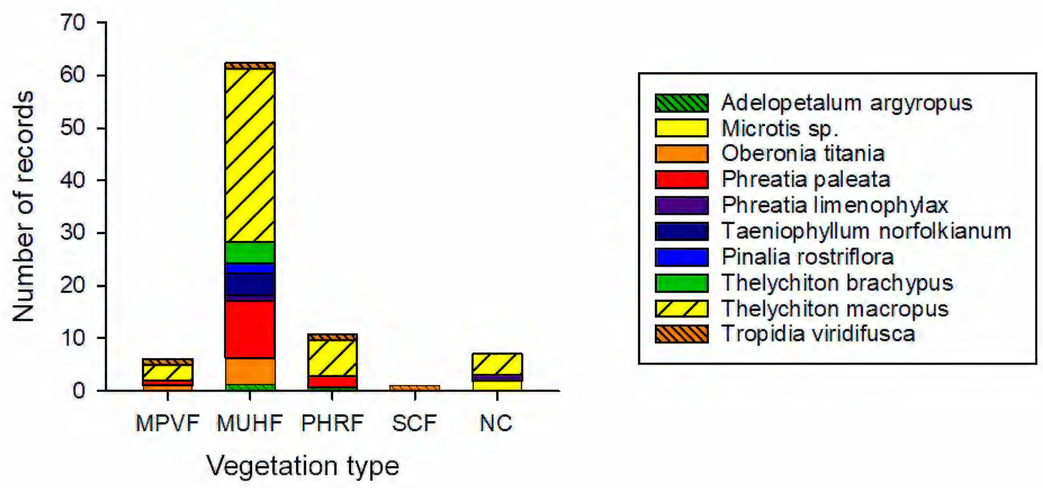

Figure 4. Orchid occurrence according to vegetation type. MPVF, Moist Palm Valley Forest; MUHF, Moist Upland Hardwood Forest; PHRF, Pine Hardwood Ridge Forest; SCF, Sheltered Coastal Forest; NC, not classified.

Discussion

Orchids are often under-represented in oceanic island floras, constituting a mean of 1.2% of plant species richness (Taylor et al. 2019). On Norfolk Island, orchids represent a higher-than-average plant species richness of 6% (11 of 190 indigenous plant species; Mills 2023). While orchids have dust-like seed that can disperse thousands of kilometres (including over oceans), many orchids are also reliant on specific environmental conditions and relationships (i.e., mycorrhizal fungi, pollinators, host trees) to survive and reproduce (Taylor et al. 2019). Both biological and biogeographic factors are likely to have contributed to Norfolk Island's orchid flora.

Biogeographic and biological inferences about Norfolk Island orchids

The unique flora of Norfolk Island comprises elements related to Australia, Lord Howe Island, New Caledonia, and New Zealand (Green 1994). Wind is the most likely mode of dispersal for orchids (Arditti and Ghani 2000), transporting seeds to Norfolk Island from neighbouring landmasses. Prevailing wind direction on Norfolk Island varies across the year. Easterly and south-easterly winds predominate in summer and autumn, shifting to south and south-westerly in winter, and southerly in spring (BOM 2022a, b). New Zealand lies to the southeast of Norfolk Island, and has 130 – 140 mostly terrestrial orchid species, including *Nematoceras acuminata* and *Thelymitra longifolia*. Most weather systems approach the Norfolk Island from the west (Mills 2023), in the direction of Australia, which has 1698 mostly terrestrial orchid taxa (including 27 species of *Microtis*), but also including the epiphytic *Adelopetalum argyropus* and *Oberonia titania*. Lord Howe Island is to the southwest and has 13 orchid species, mostly terrestrial, but also *Adelopetalum argyropus*. To the north of Norfolk Island is New Caledonia, with 209 orchid species (101 endemic) (Endemia 2022), including *Pinalia rostriflora, Phreatia limenophylax,* and *Tropidia viridifusca*. Seeds may have been carried from New Caledonia to Norfolk Island on the northerly winds that drive the cyclones that form in the more tropical waters to the north of Norfolk Island in summer months (BOM 2014).

The epiphytic orchid, *Pinalia rostriflora* was recorded on Norfolk Island for the first time during this survey and constitutes a new species record for Australian territory. *Pinalia rostriflora* is relatively widespread on New Caledonia (Endemia 2022). Eriinae, the subtribe to which *Pinalia* belongs, has been the subject of recent molecular phylogenetic research (Ng et al. 2018), highlighting phylogenetic structure and diversity within this group of orchids, which have been historically grouped together in genus *Eria*. Two other species of *Pinalia* occur in Australia, both in northeast Queensland, these are *Pinalia fitzalanii* (also in the Torres Strait area) and *Pinalia moluccana* (Jones 2021).

Despite the rich terrestrial orchid floras of neighbouring landmasses, only four terrestrial orchid species have been recorded on Norfolk Island (two species of *Microtis*, *Thelymitra longifolia*, not seen in this study; *Nematoceras acuminata*, not seen and likely extinct). The soils of Norfolk Island are basaltic, nutrient rich and well structured, but also friable and porous (DCCEEW 2021), potentially rendering them less able to support seeds of terrestrial orchids. It is also possible that introduced animals, such as cattle and pigs, may have had negative effects on terrestrial orchids, both indirectly, through vegetation degradation, and directly, through grazing, trampling, and rooting. Cattle grazing is still permitted within specific public lands on Norfolk Island, particularly on roadsides. Another explanation for the relative paucity of terrestrial orchids may lie in their requirement for a mycorrhizal symbiont. Orchid seeds are tiny and lacking in nutritive tissue and so they form a relationship with mycorrhizal fungi to provide nutrients during initial seedling development (Dressler 1981). Orchid species that form generalist mycorrhizal associations (i.e., are less species specific and/or with more widespread taxa) could be expected to be more common on islands (Taylor et al. 2019), although this is not always the case (e.g., Hawaii, Swift et al. 2019). *Microtis* species, two of which are recorded on Norfolk Island, form relationships with the relatively widespread *Tulsanella* fungi (Wei Han Lim 2015), possibly contributing to their establishment on Norfolk Island, and nearby Phillip Island. It is possible that seeds of other orchid taxa have dispersed to Norfolk Island but have been unable to establish due to the absence of appropriate mycorrhizal taxa.

Another trait common among island plant species is self-compatibility (Grossenbacher et al. 2017). Norfolk Island's endemic *Thelychiton* species (*Thelychiton brachypus* and *Thelychiton macropus*) are self-pollinating and have flowers that remain closed – they are cleistogamous. In cleistogamous species, the surface of the stigma is covered in tissue from the column, meaning that no pollen can be deposited. Because of this, each flower is self-pollinated from pollen tubes that germinate and grow directly from the anther, down to the stigmatic tissue. *Oberonia titania* is also able to self-pollinate via rain splash (Jones 2021). *Microtis* includes species that can self-pollinate and/or reproduce vegetatively (Swarts and Dixon 2009); where *Microtis* are present on Norfolk Island they occur in colonies, indicative of vegetative reproduction. *Thelymitra* species, including *Theymitra longifolia*, also have the capacity to self-pollinate (Lehnebach et al. 2005).

There were six species on Norfolk Island that had small white or pale yellow-green flowers (i.e., *Adelopetalum argyropus, Phreatia paleata, Phreatia limenophylax, Pinalia rostriflora, Taeniophyllum norfolkianum* and *Tropidia viridifusca*) – these species were also relatively rare and/or were observed to have few seed pods. This floral morphology is hypothesised to be attractive to a diverse array of small insects (Bawa 1990) and suggests pollination by generalist pollinators (cf. self-pollination). Plants can overcome pollinator limitation on islands by selecting floral traits that attract a wider variety of pollinators (Barrett 1996; Taylor et al. 2019). *Adelopetalum* is likely pollinated by flies (as are many Bulbophyllinae, Ridley 1890, Teixeira et al. 2004), while *Taeniophyllum* species are usually pollinated by bees or beetles (short spurred species of Aeridinae, Topik et al. 2005). *Tropidia viridifusca* has the fragrance of decomposing material (M. Clements pers. obs.), so is likely to attract a fly pollinator (Christensen 1994). The insect pollinators of *Phreatia* and *Pinalia* are unknown.

Extinction risk assessments of orchids on an oceanic island

Updated species extinction risk assessments are recommended for three already listed threatened species, where their only occurrence within the jurisdiction of the Commonwealth of Australia's Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) is on Norfolk Island: *Phreatia paleata* (currently EN), *Taeniophyllum norfolkianum* (currently VU), and *Thelychiton brachypus* (currently EN) (Table 2). Additionally, we recommend threatened species extinction risk assessments for five previously unassessed species/populations: *Adelopetalum argyropus* (Norfolk Island population), *Oberonia titania* (Norfolk Island population), *Pinalia rostriflora* (new, only occurrence on Australian territory), *Thelychiton macropus* (endemic to Norfolk Island, only occurrence on Australian territory), and *Tropidia viridifusca* (only occurrence on Australian territory).

We note that Takhtajan (1969) in his seminal work 'Flowering Plants, Origin and Dispersal' argued that the flora of Norfolk Island differed strongly from that of Australia (and Lord Howe Island). Moreover, the interim biogeographical regionalisation for Australia (IBRA) places Norfolk and Lord Howe Island together, and distinct from continental bioregions (Commonwealth of Australia 2012), while in marine bioregionalization, Norfolk Island is placed on its own (Australian Government 2005). In this study, we emphasise the significance of these orchid species records in the context of Australian Orchidaceae, as Norfolk Island is under the jurisdiction of Australian Government's environmental legislation (the EPBC Act), and one of the intentions of this study is to inform the conservation of these species.

A species or population limited to Norfolk Island, because of the small size of the island, may be eligible to be listed as Critically Endangered under IUCN Red List Criterion B, because the Critically Endangered threshold for extent of occurrence is <100 km^2, and the likelihood of genetic exchange outside Norfolk Island is very low, given its remoteness. Likewise, these populations may be considered severely fragmented, as all individuals are encapsulated by these small populations, isolated on Norfolk Island, and therefore, if they went extinct, there would be no possibility of recolonisation.

Determining which records are associated with which species can be challenging when the morphological and genetic boundaries among species are unclear (e.g., because of taxonomic uncertainty). However, conservation assessments often – and must – continue in the face of uncertainty (Bland et al. 2017, e.g., Melville et al. 2021). In some cases, it may be appropriate to assess populations or evolutionary significant units (*sensu* Coates et al. 2018). As Norfolk Island is clearly delimited by ocean, and orchid populations are unlikely to be undergoing genetic exchange outside Norfolk Island, we suggest assessment of the Norfolk Island populations of *Adelopetalum argyropus* and *Oberonia titania* (which, as currently circumscribed, also exist on the Australian mainland and elsewhere).

Extinction risk assessments of orchids on an oceanic island: threats

The most pervasive threat to orchids on Norfolk Island is decreased rainfall. Mean annual rainfall on Norfolk Island declined 11% between 1970 and 2020 (CSIRO 2020). Current climate change projections for Norfolk Island include a 1.3°C increase in temperature (10^{th} to 90^{th} percentile range, 1.1°C to 1.7°C) and a 6% decrease in rainfall (10^{th} to 90^{th} percentile range, -13% to +4%) by 2050 (CSIRO, Managers of World Heritage Properties in Australia and Indigenous Reference Group 2021), with possible impacts of climate change including changes in seasonal rainfall patterns, and long runs of dry years impacting on the hydrology of Norfolk Island (CSIRO 2020). Hotter, drier conditions may lead to physiological stress on and reduced recruitment of plants, and to an increased risk of fire and consequent impacts on fire-sensitive native plants and wet rainforest ecosystems (DCCEEW 2023).

Climate change poses a particular risk to the biodiversity of oceanic islands because of their many rare and endemic

species, low habitat availability and low functional redundancy (Harter et al. 2015). Moreover, epiphytes are particularly vulnerable to climate change because they exist at the vegetation-atmosphere interface (Zotz and Bader 2009). In addition, because they lack direct access to soil water, epiphytes are reliant on moisture that they intercept from the atmosphere (clouds, fog, rain) and stemflow from their host (Mendieta-Leiva et al. 2020). Epiphytes have been identified as possible indicators for floristic response to climate change (Benzing 1998) and in line with this, epiphytic orchids may provide a useful indicator of climate change impacts on the vegetation of Norfolk Island.

The majority of Norfolk Island's epiphytic orchid records were restricted to moist upland hardwood forest. The impacts of decreased rainfall on Norfolk Island's orchid flora, particularly the montane epiphytic orchids, are difficult to predict because of the many factors that influence fine-scale spatial variability in rainfall. The amount of water received by the epiphytic orchids, their forest habitat, and tree hosts, is likely to be affected by duration of cloud immersion (Scholl et al. 2007), which can occur over a longer duration than rainfall. This may result in these higher-elevation areas (including many epiphytes and hosts) receiving more precipitation than lower-elevation areas – although an overall decline may still be experienced. The forest on Norfolk Island is probably important for trapping passing cloud, providing a moist and dense canopy (storage capacity of the canopy is about 2.5 mm), and reducing sunlight and wind (i.e., moist/humid environment) (McJannet et al., 2023). These systems are complex, and while broad-scale trends are known, further research is required for robust predictions of impacts on finer spatial scales (Harter et al. 2015). Moreover, we acknowledge that our search effort was biased towards the mountain slopes of Norfolk Island National Park, which also include the greatest area of moist upland hardwood forest; future systematic survey across all vegetation types would be beneficial.

There are five orchids on Norfolk Island largely limited to moist upland hardwood forest on Norfolk Island: *Adelopetalum argyropus, Oberonia titania*, *Phreatia limenophylax* and *Taeniophyllum norfolkianum* and *Thelychiton brachypus*. The extent to which these species will be (or are already) impacted by desiccation will be influenced by their ability to avoid or tolerate drought. For example, species in the genus *Taeniophyllum* are considered drought resistant, because their leaves have (and hence water loss via stomata has) been radically reduced, with leaves remaining only as tiny papery bracts. Instead, photosynthesis occurs in the roots of *Taeniophyllum,* which contain cortical stomatal complexes enabling controlled gas exchange (Carlsward et al. 2006). *Adelopetalum* species, employing a different tactic for drought resistance, have capacity for water storage via pseudobulbs (Ramesh et al. 2020). Each pseudobulb of *Adelopetalum argyropus* is terminated by a small hard leaf, which can be deciduous, and hence provide a further water saving mechanism. Pseudobulbs of *Adelopetalum argyropus* can also become flaccid with loss of moisture, but they can recover and re-shoot after rain (M. Clements, pers. obs.). *Phreatia limenophylax* similarly stores water, through its thickened/succulent leaves (Zhang et al. 2018). Pseudobulbs and thickened cuticles are two key strategies in epiphytic orchids for maintaining water balance (Yang et al. 2016). *Oberonia titania* comprises a stem covered with succulent, equitant (folded and encasing the base of the next leaf) leaves. Much like *Adelopetalum argyropus*, *Oberonia titania* plants can become flaccid in dry conditions and recover after rainfall (M. Clements, pers. obs.). While each of these five orchids has some capacity for drought tolerance/avoidance, the extent to which this will enable persistence in the face of long-term declines in moisture availability, especially alongside predicted increases in temperature (and evapotranspiration), is unknown.

Recommendations

Investigate the taxonomic status of Norfolk Island's endemic orchids

Morphological and ecological examination indicates that the population of *Adelopetalum argyropum* may constitute a distinct species, endemic to Norfolk Island. Further taxonomic research, guided by molecular analysis, is needed to confirm the identities of the Norfolk Island *Microtis* species. Species in the genus *Microtis* pose a particular challenge for identification and delimitation, not only because of the superficially similar appearance of species and the very small size of their flowers (<5 mm), but also because of limited research to date on their molecular phylogenetics. It is likely that there are many more species of *Microtis* yet to be described (Jones 2021). Molecular taxonomic analysis will also assist in confirming whether the *Thelymitra* species (collected by de Lange in 1998, not seen on during this research) is *Thelymitra longifolia*. Lastly, further research is needed to confirm the taxonomic status of Norfolk Island populations of *Oberonia titania* (Jones 2021) and *Phreatia limenophylax* (Endemia 2016) compared to continental Australian and New Caledonia populations, respectively.

Thelychiton brachypus and *Thelychiton macropus* are endemic to Norfolk Island, though the extent that they are distinct from one another is unclear – as individuals with morphological characters intermediate/between the two species were recorded. Further research into these species is warranted. Recommendations are summarised in Table 3.

Table 3. Summary of management recommendations for conservation of orchids on Norfolk Island.

Objective	Key actions
Resolve the taxonomic and conservation status of the orchids of Norfolk Island	Resolve taxonomic status of *Adelopetalum argyropum* population on Norfolk Island, with respect to populations on Lord Howe Island and continental Australia
	Resolve taxonomic status of *Microtis* species on Norfolk Island
	Resolve taxonomic status of *Thelymitra longifolia* record on Norfolk Island, with respect to other *Thelymitra* species in Australia, New Caledonia and New Zealand
	Resolve taxonomic status of *Oberonia titania* on Norfolk Island, with respect to continental Australian populations
	Resolve taxonomic status of *Phreatia limenophylax* on Norfolk Island, with respect to records on New Caledonia
	Resolve species delimitation of *Thelychiton brachypus* and *Thelychiton macropus* from one another
Increase understanding about the population trajectories of orchids on Norfolk Island population trajectories	Conduct a systematic survey of the Norfolk Island to locate any orchid records missed in this study
	Monitor a subset of permanently marked individuals (abundant species)
	Monitor all individuals (rare species)
	Develop detailed monitoring program with stratified sampling across environmental conditions to identify correlates with decline
Protect and restore Norfolk Island's orchid species	Prioritise moist upland hardwood forest (MUHF) for protection, and MUHF tree species for restoration planting
	Conduct research to determine preferred host tree species and microhabitats for Norfolk Island orchids
	Conduct research to inform *ex situ* conservation of Norfolk Island orchids, such as determining species requirements for germination, cultivation, and transfer from nursery to the field

Monitoring

Establishment of a monitoring program is important to detect decline in Norfolk Island orchid populations, especially for the epiphytic orchids predicted to be affected by climate change. For accurate inference about population decline, a sample of permanently marked individuals should be monitored for survival, growth and recruitment. Monitoring should be undertaken at least annually, but more frequently in response to disturbance including windstorms and severe drought. For many species, it may be possible to monitor all known individuals of the species (e.g., *Adelopetalum argyropus*), giving exact data on the status of the population. For other species with larger populations, a subset of individuals may be monitored. When there are many individuals, consideration should also be given to stratifying the monitored individuals by other variables which may impact survivorship, such as elevation or host tree species. With appropriate experimental design, a diagnostic monitoring program may be established such that the reasons for decline (or increase) may be identified.

Protecting and restoring Norfolk Island's threatened orchid species

Five of Norfolk Island's orchids are epiphytes with distributions centred on moist upland hardwood forest (MUHF), highlighting this vegetation type as a priority for protection and restoration. Key species in MUHF include sharkwood (*Dysoxylon bijugum*), beech (*Myrsine ralstoniae*), ironwood (*Nestegis apetala*), and native oleander (*Pittosporum bracteolatum*). Future research should include the identification of the tree species and associated micro-environments that compose the habitats for the orchid species, such that appropriate conditions for epiphytic orchid establishment can be considered in the ongoing forest restoration work on Norfolk Island.

Management of introduced animals may also assist with orchid conservation. Terrestrial orchids should be protected from grazing during flowering – with careful consideration of issues of competition with other plants, especially exotic grasses, if fencing is erected. Rodent predation of seed has also been hypothesised as a threat to several species, including *Phreatia paleata* and *Thelychiton brachypus* (DCCEEW 2023).

Ex situ conservation may also be used to mitigate extinction risk. However, knowledge of *ex situ* conservation methods for epiphytic orchids is fragmentary, and reports in the scientific literature are few (e.g., Zettler et al. 2007). Moreover, understanding of the requirements for successful translocation of epiphytic orchids from the wild to cultivation and/or new wild sites remains limited (although see Yam et al. 2011, Izuddin et al. 2018, Izuddin et al. 2019). Anecdotally, rescued branch fall epiphytic orchid species are difficult to maintain *ex situ* (M. Clements pers. obs.). Swarts and Dixon (2009) mention the apparent generality of mycorrhizal associations in some epiphytic orchids, allowing them to be grown in large-scale horticulture – although they note that other species appear to be more specific. Nevertheless, the limited information available gives guidance for growing some groups from seed, such as *Thelychiton* species (sometimes placed in the broader genus

Dendrobium) (e.g., Teixeira et al. 2015), whereas others are likely to be more difficult (*Adelopetalum, Oberonia, Phreatia* spp.). Further research is needed, not only in determining germination requirements, but in cultivation through to successful transfer from the nursery to the field. In summary, possible management actions include: the creation and improvement of habitat for orchids, including planting of host trees in suitable locations; seed banking and *ex situ* cultivation (of all species, particularly the rarest species) to create insurance populations and stock for translocations; and translocation, consisting of augmentation and/or population establishment. Further research is needed to determine the relative importance of, and optimal methods and/or locations for implementing, all of these options.

Conclusion

Norfolk Island has at least 11 orchid species, constituting a relatively high proportion of its native plant flora. Potential explanations for this richness are manifold but may include the proximity of multiple, species-rich sources of orchid seed, diversity of microclimates, and the availability of traits such as self-pollination and associations with generalist pollinators and mycorrhizae. Norfolk Island is currently recognised as having three or four endemic orchid species (*Taeniophyllum norfolkianum, Thelychiton macropus*, *Thelychiton brachypus,* and in some assessments *Phreatia limenophylax*); in addition, it has the only occurrences on an Australian territory for at least three more species (*Phreatia paleata*, *Pinalia rostriflora*, and *Tropidia viridifusca*). Because of the remoteness of Norfolk Island, genetic exchange with populations external to Norfolk Island is likely to be limited, hence further investigation of the morphological and genetic distinctiveness of these species is required, along with the Norfolk Island populations of *Adelopetalum argyropus* and *Oberonia titania*. The epiphytic orchids of Norfolk Island are largely centred on the moist upland forests – consideration must be given to the vulnerability of these species to climate changes, especially in the context of the significant decreases in annual rainfall already recorded. A robust monitoring program is essential to further understand the range and population trends of orchid species on the island. *Ex situ* cultivation, translocations to new sites and restoration plantings may assist in ensuring the long-term persistence of Norfolk Island's unique orchid flora.

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Appendix 1: Orchid scientific names, synonyms and common names.

Species	Synonyms	Local name	Other common names
Adelopetalum argyropus	*Bulbophyllum argyropus*[1]	One Leaf Orchid	Silver Strand Orchid[2]
Nematoceras acuminata	*Corybas acuminatus*[1]	None	Dancing Spider Orchid[2]
Microtis sp.	None	None	Onion Orchid
Oberonia titania[1]	*Oberonia palmicola, Oberonia neocaledonica*	Norfolk Island Oberonia	Soldiers Crest Orchid[2]
Phreatia paleata	*Eria paleata*[1]	None	White Lace Orchid[2]
Phreatia limenophylax	*Plexaure limenophylax*[1]	Norfolk Island Phreatia	Norfolk Island Caterpillar Orchid[2]
Pinalia rostriflora	None	None	None
Taeniophyllum norfolkianum[1]	*Taeniophyllum muelleri*	Minute Orchid Taeniophyllum	Norfolk Island Ribbon Root[2]
Thelychiton brachypus	*Dendrobium brachypus*[1]	Short-caned Orchid	Stubby Cane Orchid[2]
Thelychiton macropus	*Dendrobium macropus*[1]	Long-caned Orchid	Norfolk Island Cane Orchid[2]
Thelymitra longifolia	None	None	Sun Orchid
Tropidia viridifusca[1]	None	Ground Orchid	Dark Crown Orchid[2]

[1] Name accepted on the Australasian Plant Census https://biodiversity.org.au/nsl/services/search/taxonomy;

[2] Name sourced from Jones (2021).

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A review of the fire response of *Genoplesium* R. Br. *sensu lato* (Orchidaceae) and implications of fire management on habitat

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***Abstract*:** *Genoplesium* R.Br. *sensu lato* is an Orchidaceae genus of perennial geophytic herbs with up to 80 recognised species primarily distributed in eastern Australia. Species are commonly found occupying relatively open habitats, including partially cleared habitats such as track margins, powerline easements and bushfire protection zones. In the absence of fire and other disturbances, dense vegetation commonly appears to outcompete the genus. Habitat management for threatened *Genoplesium* species has involved regular slashing of habitat to control vegetation regrowth, although this management action has only been applied across relatively small areas of habitat.

Despite a lack of detailed studies on *Genoplesium* species, there is a large body of evidence from opportunistic observations and studies of related terrestrial orchid species which suggests that mature individuals of *Genoplesium* are likely to be stimulated by fire during their dormancy periods. Observed increases in the size of monitored populations in the season immediately after fire indicates that members of the genus may be dormant, or at least undetectable, in unburnt habitat until fire or similar disturbance triggers increased flowering. The exclusion of fire from habitat is likely to result in reduced flowering, and recruitment, and ultimately may cause localised extinctions.

The fire histories of two short range endemic species *Genoplesium insigne* and *Genoplesium branwhiteorum* on the NSW Central Coast provide a case study.

Detailed field studies for many rare *Genoplesium* species including targeted post-fire surveys are required to confirm their fire responses, and to improve understanding of their potential to remain dormant in unburnt habitat, to investigate the impact of fire exclusion and to inform conservation assessments and land management practices.

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Introduction

Genoplesium R.Br. *sensu lato* is a genus of perennial geophytic herbs with up to 80 recognised species (Copeland and Backhouse 2022). The genus is primarily distributed in southern and eastern Australia, with a single species known from New Caledonia and two species occurring in New Zealand (Jones 2001). The accepted taxonomic treatment of this genus differs between jurisdictions within Australia, see Renner et al. (2022) for a discussion of the various treatments, although for the purposes of this review the generic concepts of Clements and Jones (1989) are applied, consistent with the current position of the National Herbarium of New South Wales. *Genoplesium* is a member of the Prasophyllinae subtribe within the Orchidaceae and is distinguished by a hinged and mobile labellum, a cushion like callus on the labellum and deeply notched column wings (Jones 2001). Other characteristics of the genus include a short, fused leaf and peduncle on flowering plants with small, often dark coloured flowers, which can make detection of individuals in the field difficult.

A comparatively large proportion of *Genoplesium* species are recognised as rare or threatened with up to 18 species listed under relevant state legislation across Australia. Species of *Genoplesium* are commonly detected in areas with an open vegetation structure, having a low cover of canopy and midstorey species, such as open rock plates (Jones 2021) and areas which have been partially cleared, or thinned, such as track margins, powerline easements and bushfire protection zones (Jones et al. 1999; Bernhardt et al. 2017). In some cases, dense regrowth of native vegetation within previously open habitat has resulted in a decline in flowering and detection of rare *Genoplesium* species, a pattern observed across related terrestrial orchids in Australia (Lunt 1997) and orchid species in Europe (Köhler et al. 2023). Consequently, ongoing control of vegetation regrowth has been implemented or recommended for management of habitat for several rare *Genoplesium* species including *Genoplesium insigne* (DPE 2023a), *Genoplesium branwhiteorum* (DPE 2023b), *Genoplesium firthii,* (DPIWE 2002), *Genoplesium brachystachyum* (TSS 2016), and *Genoplesium morrisii* (TSS 2013). This biomass control, commonly involving annual slashing of vegetation outside of the active growth season of the *Genoplesium* species, is effective for maintaining population sizes of rare *Genoplesium* species across periods of up to five years (Towle et al. 2022). However, there are limitations on the area across which this management action can be implemented, and its effectiveness appears to be dependent upon regular ongoing slashing which requires ongoing resources, funding, and landowner support. Further, the long-term implications of regular slashing are unknown, and it has been hypothesised that frequent stimulation of flowering could divert resources away from development of replacement tubers (Weston et al. 2005). Due to these limitations, there is a need for more long-term and widely applicable habitat management practices.

Fire, which has influenced the structure and composition of vegetation in Australia for millennia (Miller and Murphy 2017), may represent a more long-term and widely applicable solution to maintenance of an open habitat structure, without the regular stimulation to flower which may divert resources away from replacement tuber production. However, the response of *Genoplesium* species to fire often remain unknown or poorly understood. For example, under the NSW Bushfire Environmental Assessment Code (RFS 2021), more than half the *Genoplesium* species listed on the NSW 'Threatened Species Hazard Reduction List' (RFS 2013) have controls prohibiting fire, slashing or trittering within 100 m of the known location of the listed rare *Genoplesium* species. The aim of this review is to synthesise existing knowledge of the fire response of *Genoplesium* species to inform future research and habitat management priorities.

Accounts of the fire response of *Genoplesium* plants

There are multiple accounts of increased flowering of *Genoplesium* species in the flowering seasons after fire. A large increase in the population of *Genoplesium superbum* was reported in areas of Morton National Park following bushfire in early 2020, although large increases were also detected in unburnt populations across the same period which was attributed to increased rainfall (Canackle et al. 2020; Roper 2021). Population counts of *Genoplesium ectopum* following bushfire in January 2003 also indicated that high intensity fires are likely to have benefited the species, although the fire response was also considered to be influenced by subsequent rainfall (Frawley 2008). Large populations of *Genoplesium ruppii* and *Genoplesium fimbriatum* have been detected for the first-time following fires within Northern Sydney (APS 2016) and on the NSW Central Coast (Ecoplanning 2020). Additionally, multiple orchid field guides report that *Genoplesium* species as a whole are fire stimulated (Jones et al. 1999; Jones 2021), or identify individual species as growing, or flowering, prolifically after fire including *Genoplesium archeri*, *Genoplesium morrisii* and *Genoplesium pumilum* (Jeanes and Backhouse 2006).

While there are multiple accounts of positive responses of mature *Genoplesium* species to fire, not all reports are positive. Populations of *Genoplesium despectans* within Kinglake National Park were not seen following the Black Saturday Bushfires in Victoria in 2009 (Duncan 2012). The same species was observed flowering at pre-fire levels in Bunyip State Park following the same fire event, indicating that the response of the species varied between populations (Duncan 2012). The varied responses to fire within and between *Genoplesium* species indicates that not all fire events are equal and that many variables may influence the response. The influence of seasonality of fire on the response of *Genoplesium* species has not been studied, however it has been shown to have a large influence on the fire response of terrestrial orchid genera including *Thelymitra*, *Pterostylis* and *Glossodia* (Jasinge et al. 2018). Fires during the active growing and flowering stages of these genera were found to have a deleterious impact on studied populations, while fires during dormancy periods did not impact populations in the same way (Jasinge et al. 2018). Fire intensity may

also influence the response of *Genoplesium* species. Fire stimulated flowering has been reported from high intensity fires including the Morton National Park fires in early 2020 (Canackle et al. 2020) and low intensity prescribed burns (Ecoplanning 2020).

Post-fire rainfall has also been identified as impacting the fire response of *Genoplesium* species (Frawley 2008; Canackle et al. 2020;). However irrespective of fire, rainfall is identified as both determinant of flowering and influencing the specific timing of flowering within a season (Bower et al. 2015; Canackle et al. 2020; Roper 2021). Therefore, any stimulated flowering of *Genoplesium* from fire is likely to be dependent upon follow-up rainfall.

Fire response of seed and immature *Genoplesium*

While documented accounts of the fire response of *Genoplesium* species do not distinguish between mature and immature plants, observations of *Genoplesium* post-fire commonly refer to enhanced flowering and are therefore only observing the response of mature individuals. Detailed studies and observations of fire impacts on the immature stages of *Genoplesium* are lacking. Seeds of *Genoplesium* species, and indeed almost all orchids, are minute and dust like (Rasmussen 1995). The simple seeds of *Genoplesium* species do not possess a hardened seed coat, or other adaptations, which may protect seeds from fire. Therefore, seeds of *Genoplesium* species are almost certainly killed when exposed to the heat and steam produced by fire.

While seed exposed to fire will almost certainly be killed, seed released in a post fire environment may be positively impacted by recent fire events. In addition to increased germination rates, a range of evolutionary advantages have been hypothesised for fire stimulated flowering and seed release into post-fire environments. This includes optimal levels of resources required for both growth and reproduction of plants (water, nutrients, space, and/or light), reduced competition for these resources from fire-killed species, reduced risk of reproductive failure from further fires, and more effective wind dispersal as the surrounding vegetation has yet to close over again (Lamont and Downes 2011).

The response to fire of protocorms and immature tubers of *Genoplesium* species also remain unstudied. However, as these life stages occur underground, the response is likely to depend upon the degree of insulation from heat provided by the substrate in which individuals are growing. There is anecdotal evidence of fire negatively impacting immature seedlings with multiple fires in quick succession having been identified as being detrimental to immature orchids germinating after the initial fire (Jones 2021).

Dormant until disturbed?

The short time-frame between fire events and observed increases in flowering of *Genoplesium* species (less than 12 months) indicates that enhanced flowering in the first season after fire is the result of mature individuals being stimulated to flower and does not represent post-fire recruitment within burnt habitat (although this may occur after fire). The time to first flowering of Australian terrestrial orchid genera including *Genoplesium*, incorporating the period from seed release through protocorm development and immature tuber stages, is reported to be in the order of 2-3 years in cultivation and approximately 4-5 years in the wild (Backhouse and Cameron 2005). Therefore, any increased recruitment following fire events is unlikely to be observed in the mature flowering population for up to three years. Increases in the size of monitored populations of *Genoplesium* species in the first seasons post-fire (Frawley 2008; Canackle et al. 2020) are most likely the result of previously undetected individuals, either due to dormancy or non-flowering, being stimulated to flower by fire. Due to the highly cryptic nature of non-flowering *Genoplesium* species, failure to detect these individuals during pre-fire monitoring projects does not necessarily indicate their absence.

The possible occurrence of dormant or non-flowering *Genoplesium* individuals within unburnt habitat has implications for the conservation and habitat management of rare species within the genus. Efforts to protect only those locations where rare *Genoplesium* species have been detected through flowering has the potential to overlook large areas of habitat with potentially large dormant populations. The period after fire or slashing where flowering is enhanced remains unknown and detailed studies are required to address this question.

Fire history of two short range endemic *Genoplesium* species on the NSW Central Coast: A case study

Two rare *Genoplesium* species, *Genoplesium insigne* and *Genoplesium branwhiteorum* (**Figure 1**), have restricted distributions within the Wyong IBRA subregion (Thackway and Cresswell 1995), on the NSW Central Coast. Known occurrences of these two species are almost entirely restricted to areas which have been partially cleared, or thinned, such as track margins and areas regularly slashed for infrastructure protection such as powerline easements and bushfire protection zones. Consequently, habitat management for these species has included ongoing control of vegetation regrowth at multiple sites which has been an effective tool at maintaining population sizes across periods of up to five years (Towle et al. 2022; DPE 2022a). Enhanced flowering of both species has been observed in the season after a bushfire conforming to the general trend in *Genoplesium* of fire-stimulated flowering and dormancy in the absence of fire.

It was therefore hypothesised that the occurrence of these species almost entirely within partially cleared habitats was a result of fire exclusion from suitable habitats and the inability to detect the species within long unburnt habitat. To investigate how the recent fire history across the region may have influenced the current pattern of occurrence of these

two species, the fire history of the Wyong IBRA sub-region (NPWS Fire History Dataset, DPE 2022b) was compared to the recommended fire intervals (Kenny et al. 2004), for Plant Community Types (PCTs) mapped across the region (DPE 2022c). PCTs present within the region, but in which the species have not been previously recorded (DPE 2022d) were excluded. The recommended fire interval for those PCTs associated with *Genoplesium insigne* and *Genoplesium branwhiteorum* ranged from five to fifty years (Kenny et al. 2004). Across the Wyong IBRA subregion, a total of 37,795 ha of habitat has been mapped as supporting PCTs with which *Genoplesium branwhiteorum* and *Genoplesium insigne* are associated (DPE 2022d; **Figure 2**). For approximately 70 % (26,366 ha) of this habitat the time since last fire exceeded the recommended maximum fire interval or no fire has been recorded. Further, in approximately 85 % (32,225 ha) of the identified habitat the time since last fire exceeded the median of the recommended fire interval. While the fire history dataset used in this analysis is unlikely to be complete, the majority of habitat for these species within their restricted distribution has remained unburnt for extended periods exceeding the maximum recommended fire interval. While this doesn't confirm that fire exclusion has caused the current pattern of known occurrence for these two rare *Genoplesium* species, it highlights the potential for fire exclusion to have caused an underestimation of areas of occupancy. Detailed field studies are required to investigate the role of fire in the life cycle of these species and its role in maintaining suitable habitat for these species.

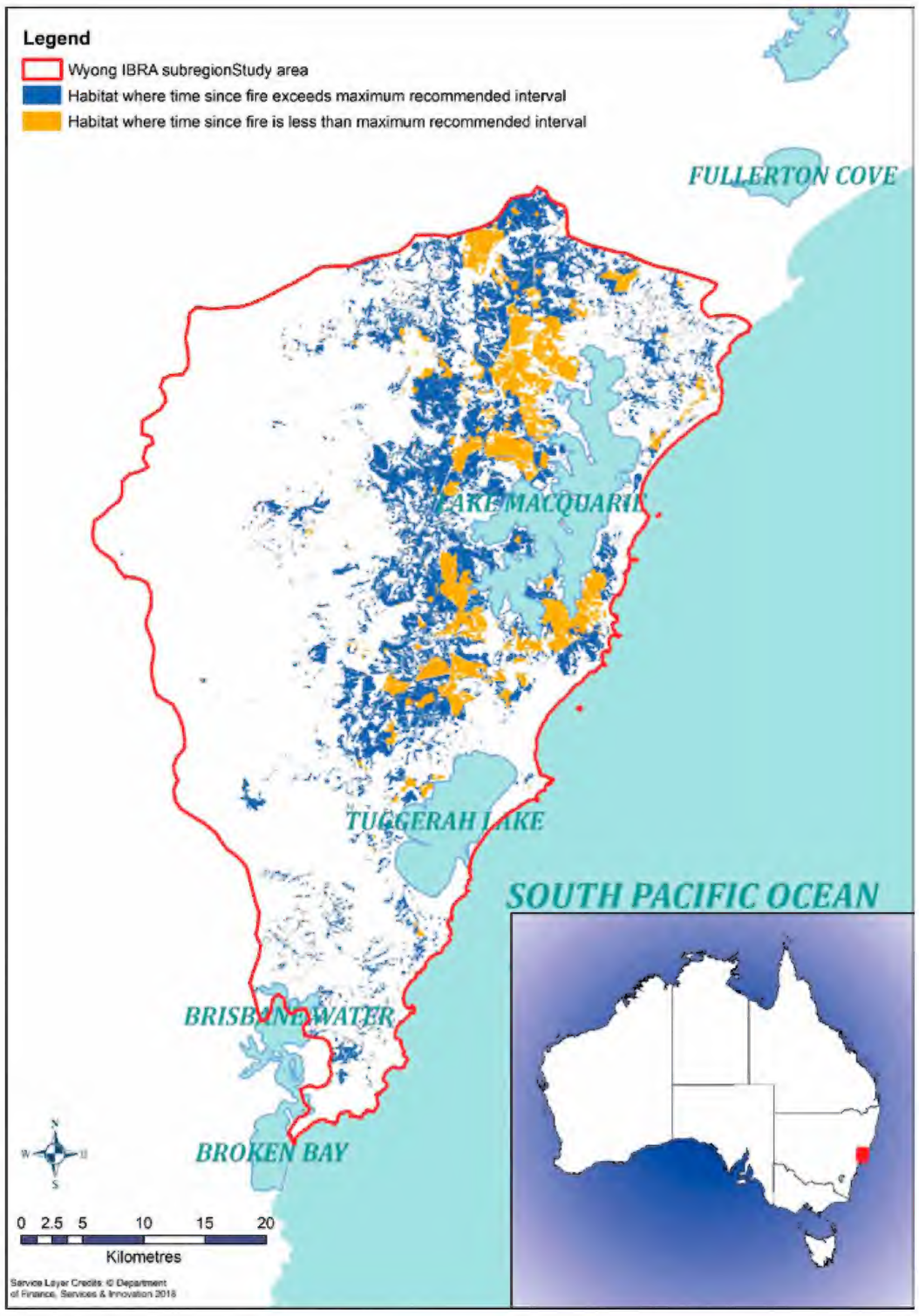
Legend
Wyong IBRA subregionStudy area
Habitat where time since fire exceeds maximum recommended interval
Habitat where time since fire is less than maximum recommended interval
FULLERTON COVE
LAKE MACQUARIE
TUGGERAH LAKE
SOUTH PACIFIC OCEAN
BRISBANE WATER
BROKEN BAY
0 2.5 5 10 15 20
Kilometres
Service Layer Credits: © Department of Finance, Services & Innovation 2018

Figure 2: Habitat where time since last fire exceeds recommended fire intervals for the mapped PCT.

Figure 1: ***Genoplesium insigne*** a) entire plant, b) inflorescence, and ***Genoplesium branwhiteorum*** c) entire plant, d) inflorescence

Conclusions

Despite the lack of detailed studies on the fire response of individual *Genoplesium* species, there is a body of evidence which suggests that fire and mechanical vegetation slashing during their dormancy periods can stimulate mature individuals of the genus to flower. However, the stimulated flowering is likely to be influenced by, or dependent upon, post-fire rainfall. The association of many *Genoplesium* species with open, slashed, or thinned habitats suggests that these species may be dependent upon fire, or other disturbance for the maintenance of suitable habitat structures. In the absence of fire, or other disturbances, these species may remain dormant, or at least undetectable. The exclusion of fire from habitat for the genus is likely to reduce flowering, seed production and recruitment and may drive population declines in some species. Implementing fire as a management tool across the habitat of rare *Genoplesium* species may reverse such declines and maintain areas of suitable habitat for these species across their range. There is a need for more detailed field studies, incorporating population monitoring before and after fire events, to confirm the hypotheses presented within this review and to investigate how individual aspects of fire which may influence the response of the genus. An improved understanding of the

role of fire in the ecology of *Genoplesium* species would better inform our understanding of the conservation status of the many rare species and land management practices for their conservation.

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Regeneration of *Eucalyptus delegatensis* R.T.Baker subsp. *delegatensis* (Alpine Ash) in Kosciuszko National Park after the severe 2003 and 2020 fires

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Abstract: *Eucalyptus delegatensis* R.T. Baker subsp. *delegatensis* (Alpine Ash) is the dominant canopy species in large areas of montane forest in the Australian Alps. As the species is killed by high severity fire, minimal intervals between fire are required for the species to germinate and to reach reproductive maturity to ensure stand persistence. In 2003 and again in 2020, extensive high severity fires occurred in southeastern Australia. These burnt through large tracts of Kosciuszko National Park in New South Wales, where the 9309 ha of *Eucalyptus delegatensis* that burnt at high severity in both 2003 and 2020 is of particular management concern.

In 2021, using an existing array of *Eucalyptus delegatensis* dominated monitoring plots set up between 2008 and 2013, we undertook a survey of seedling regeneration in plots burnt either at low severity in 2003 and high severity in 2020 or burnt at high severity in both 2003 and in 2020. We found a highly significant tendency for greater recruitment in plots only burnt at high severity 2020 as compared with plots burnt at high severity in both 2003 and 2020. Despite this significant difference, there was still robust recruitment in the plots burnt twice at high severity and there were no plots where recruitment failure was observed. In mixed stands with other re-sprouting eucalypt species present, the overwhelming majority of seedlings post-fire were of *Eucalyptus delegatensis*. For plots burnt twice at high severity, greater recruitment of *Eucalyptus delegatensis* occurred in mid-elevation stands, as compared to stands at the lower and upper limits of the elevational range of the species within Kosciuszko National Park.

Despite the downward trend in *Eucalyptus delegatensis* seedling recruitment with short inter-fire intervals, the 17 year interval between 2003 and 2020 allowed early maturing saplings to establish a viable canopy seed bank. Monitoring the growth and survival of the post-2020 cohort of seedlings will be critical to inform appropriate fire management. Our dataset makes a significant contribution to understanding recruitment and survival in eucalypts post-fire in the Australian Alpine Bioregion and it will be important to continue to document the time to first flowering and fruiting of individuals in fire affected stands and to track the proportion of saplings reaching reproductive maturity over time, to gauge stand vulnerability to further high severity fires over coming decades.

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Introduction

Eucalyptus delegatensis R.T.Baker subsp. *delegatensis* (Alpine Ash) is a forest tree growing up to 40 metres in height. This subspecies occurs in montane forest communities in south-eastern Australia from the northern Brindabella Range in New South Wales southwards into eastern Victoria through to Mt Macedon. (Boland *et al.* 2006; Brooker and Kleinig 2006), with *Eucalyptus delegatensis* subsp. *tasmaniensis* Boland occurring in central, northern and eastern Tasmania (Boland and Dunn 1985). Individuals of subspecies *delegatensis* are killed after 100% canopy scorch, but adults can survive lower intensity surface or understorey fires where canopy scorch does not occur (Bowman and Kirkpatrick 1986a). In contrast, subspecies *tasmaniensis* is a strong epicormic resprouter (Rodriguez-Cubillo *et al.* 2020).

In New South Wales (NSW), *Eucalyptus delegatensis* is found primarily on steep to moderately sloping terrain across a wide range of aspects, occurring both in monospecific stands and in association with other eucalypts, particularly *Eucalyptus dalrympleana* subsp. *dalrympleana* (Mountain Gum) and *Eucalyptus pauciflora* (Snow Gum). Other less common associates are *Eucalyptus robertsonii* subsp. *robertsonii* (Robertson's Peppermint), *Eucalyptus dives* (Broad-leaved Peppermint), *Eucalyptus viminalis* (Manna Gum) and in northern areas *Eucalyptus fastigata* (Brown Barrel) (Costin 1954; Gellie 2005). The understorey found in *Eucalyptus delegatensis* stands varies from shrubby to grassy, with common high cover shrub species including *Daviesia mimosoides* subsp. *mimosoides, Daviesia latifolia, Daviesia ulicifolia, Acacia obliquinervia, Coprosma hirtella* and *Polyscias sambucifolia*. Herb and grass layers typically have a high cover of *Lomandra longifolia, Stellaria pungens, Asperula scoparia, Viola betonicifolia* and *Poa phillipsiana*.

Eucalyptus delegatensis dominated forests occupy approximately 105,000 ha of NSW, extending over 150 km from the Victorian border, through Kosciuszko National Park (KNP) and Bimberi Nature Reserve to Brindabella National Park, with the northern limit occurring immediately north of Mt. Coree. Within KNP, *Eucalyptus delegatensis* dominated forests occupy approximately 77,050 ha, representing 73% of the NSW extent of the species (Figure 1). Most stands of *Eucalyptus delegatensis* in KNP occur on slopes in the montane zone between 1000 m and 1400 m elevation, but the species can occur up to 1750 m elevation into the subalpine zone. Annual average rainfall across *Eucalyptus delegatensis* stands in KNP ranges from 850 mm-1700 mm per annum with an annual average temperature range of approximately 6.5^{0}C -11.5°C. Most stands occur on plutonic rock types, but the species can also occur on volcanic and sedimentary geology.

Being fire-interval sensitive and subject to 'interval squeeze' (Enright *et al.* 2015), *Eucalyptus delegatensis* is likely to be adversely impacted by changes in climate and fire regimes (Bowman *et al.* 2014). In Victoria in 2003, several stands were fire-killed that had either been burned twice or logged in the preceding 20 years. These were predicted to mostly return as shrublands dominated by *Acacia* species, and a major effort was undertaken to reseed many of these areas, at a cost of around $1000 per hectare (Bassett 2013; Bassett *et al.* 2015; Fagg *et al.* 2013). However, it is unclear how much of the affected areas had no *Eucalyptus delegatensis* regeneration in the absence of aerial seeding. Boland (1985) raised a cautionary note regarding re-seeding and the potential for hybridisation between different provenances affecting the genetic integrity of natural populations, an issue first raised by Pederick (1976). At this stage, in the case of stands in KNP and adjacent reserves, no artificial re-seeding has been undertaken and the current management and monitoring regime which we outline below can be regarded as a 'natural experiment' (Diamond 1983; Green 2009).

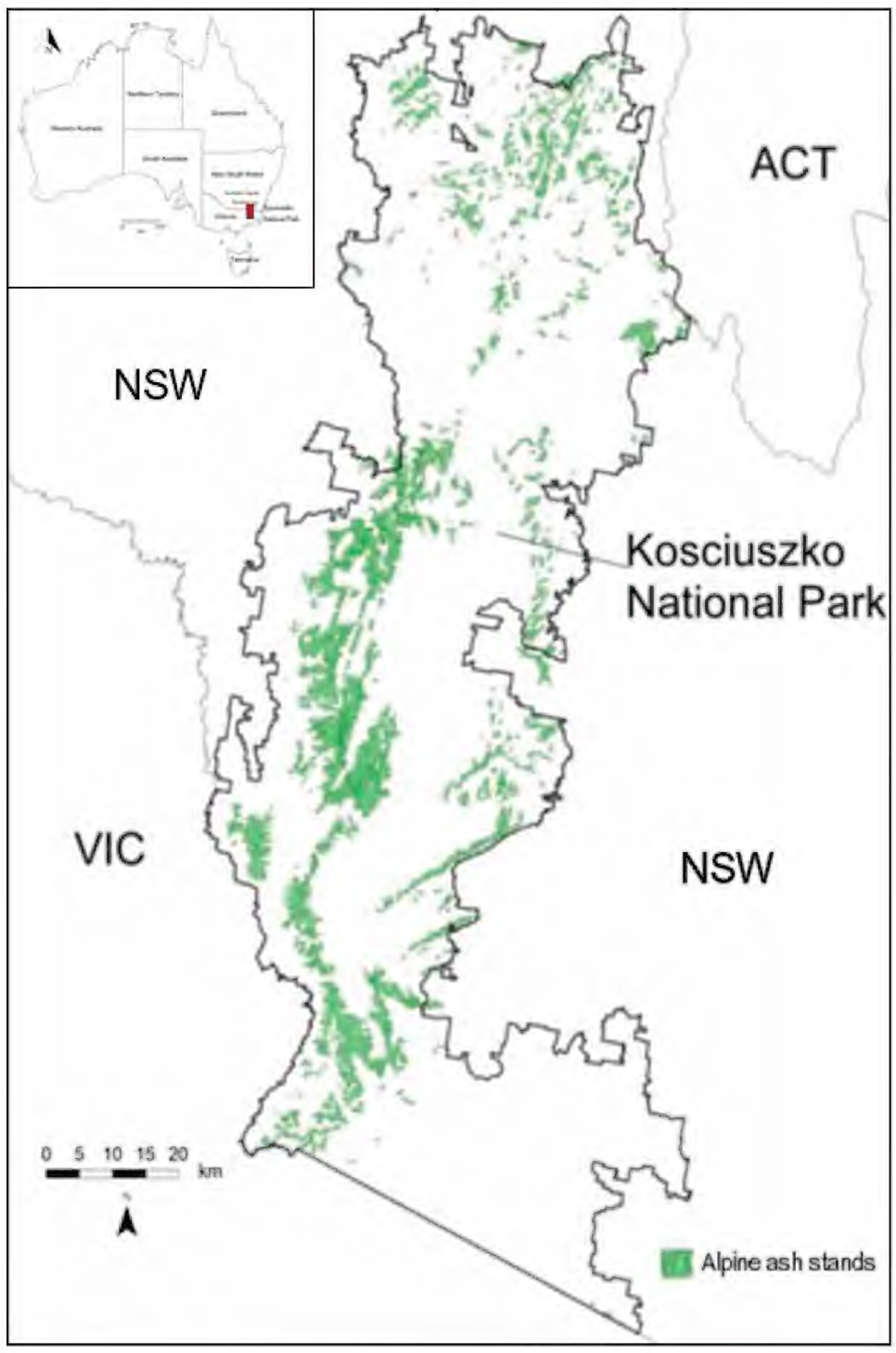

Figure 1. Distribution of Alpine Ash dominated stands in Kosciuszko National Park (Map: NSW Department of Planning & Environment).

KNP occurs in the Australian Alps bioregion (Thackway and Cresswell 1995) which is contained within the broader Southern Tablelands region of NSW. Climate change predictions for the Southern Tablelands are for a decline in rainfall, particularly in winter (NSW Office of Environment and Heritage 2014). Rainfall in winter is predicted to fall between 20-40%, with declines of up to 17% in spring and autumn, but an increase of between 18-29% in summer. The expected decline in winter rainfall is more significant than the increase in summer rainfall since most of the rain in the Australian Alps falls during the cooler months with the passage of cold fronts. It is the southward shift of these fronts that is expected to result in reduced rain and snowfall. Temperatures, both daily maxima and minima, are forecast to be 1.0 -2.5°C higher in all

seasons. Drier spells in the southern tablelands are likely to occur more often and there is likely to be an increased number of days of extreme fire danger. Severe and extensive wildfires usually occur after these conditions as for example occurred in 1926, 1939 and 2003 fires, which were each preceded by long periods of below average rainfall (Zylstra 2006). Such conditions are expected to become more frequent (Cai *et al.* 2014; Hennessy *et al.* 2005; Lucas *et al.* 2007).

Recent Fire history in Kosciuszko National Park

The most significant fire events in KNP since the 1939 fires (Zylstra 2006) are the large landscape fire events that occurred in 2003 and in 2020. While the majority of montane eucalypt species found within KNP resprout after even high severity fire, *Eucalyptus delegatensis* stands, being fire-killed, were significantly affected by these two fire events both singly and in combination.

In January 2003, approximately 49,500 ha (64.2%) of the 77,050 ha occupied by *Eucalyptus delegatensis* dominated stands in KNP was burnt. Fire severity mapping produced after these fires showed that of the 49,500 ha which burnt, 28,625 ha was burnt at high severity with 100% crown scorch, with the remaining 20,875 ha burnt at low severity without crown scorch (Barrett 2006). After the 2003 fire event, 37% of *Eucalyptus delegatensis* stands in KNP were in a fire-killed state with regeneration from canopy-stored seed required to re-establish stands. Seventeen years later in January 2020, the Dunns Road and northern Hume Complex fires burnt into the mid-northern and south-western parts of KNP (Figure 2) burning through approximately 25,100 ha (32.5%) of the area occupied by *Eucalyptus delegatensis* in the park. In the 2020 fire event, 19,077 ha burnt at high severity and 6,023 ha burnt at low severity. Of the total extent of *Eucalyptus delegatensis* in KNP, 25% burnt in both 2003 and in 2020.

However, because low severity fires do not kill adult *Eucalyptus delegatensis* trees, the area burnt on its own does not reflect the state of maturity and short-term fire sensitivity of *Eucalyptus delegatensis* stands.

The critical areas of concern are those stands that have burnt at high severity resulting in tree death either in 2003 or in 2020 and particularly stands that burnt at high severity in both fire events. The combined effects of the 2003 and 2020 fires in KNP have resulted in areas which fall into three categories:

a) areas containing old mature trees that may be either long unburnt or which have only burnt at low severity in 2003 and/or in 2020,

b) areas containing maturing sapling regrowth from the 2003 fires which did not burn in 2020,

c) seedling regeneration in areas which burnt at high severity in both 2003 and in 2020, or at high severity only in 2020.

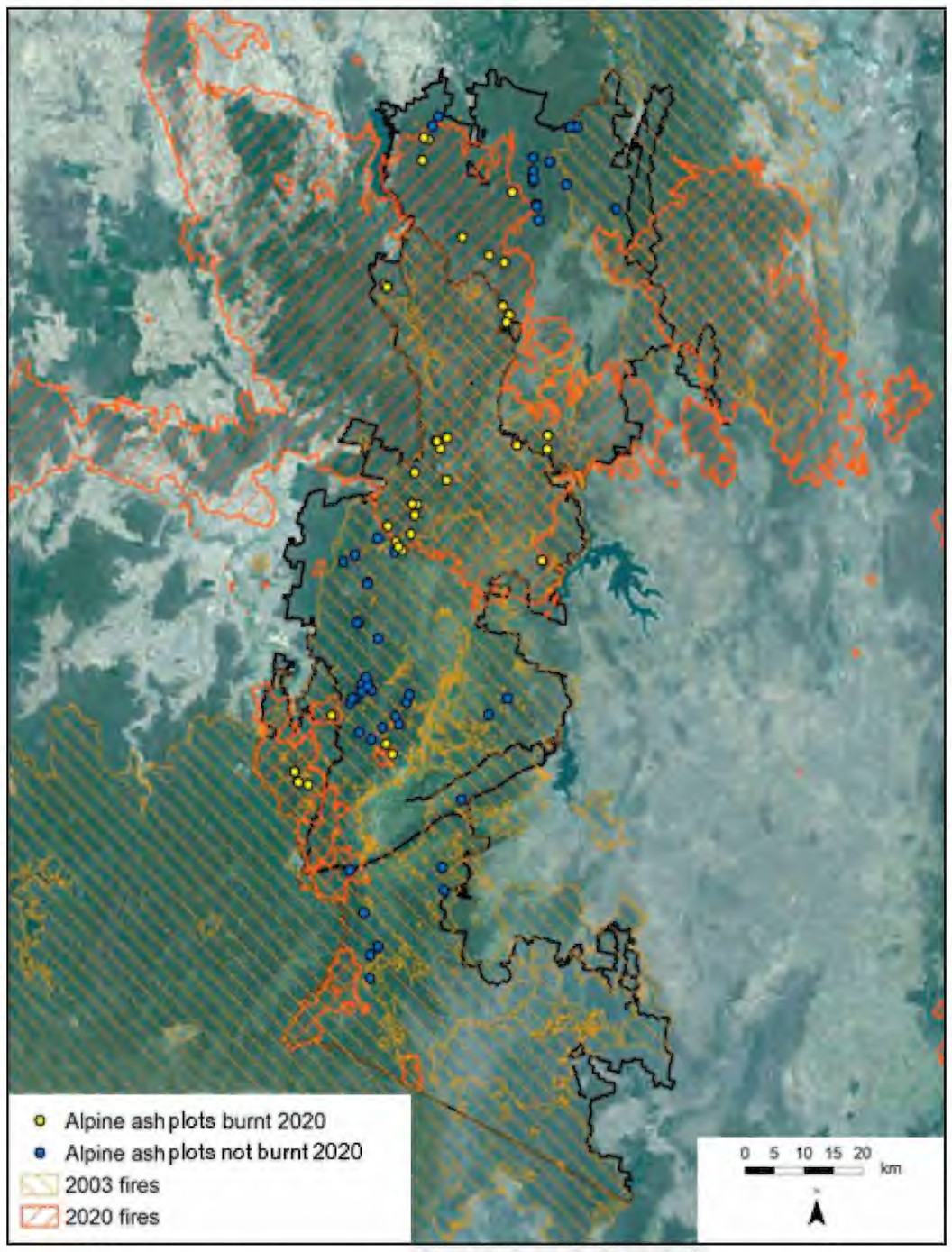

Figure 2. Alpine Ash monitoring plots in relation to the extent of the 2003 and 2020 fires in Kosciuszko National Park (Map: NSW Department of Planning & Environment).

A shift has occurred in these categories from 2003 to 2020, with only 38% of stands currently in a mature state (i.e. unburnt in 2003 and 2020, or else burnt in fires of low intensity which did not affect the canopy) and 62% of stands currently being either maturing saplings or recently germinated seedlings (Table 1).

Although the 2003 and 2020 fires had different footprints, of particular concern is the 9309 ha of *Eucalyptus delegatensis* that burnt at high severity in both 2003 and in 2020 (crosshatching in Figure 2). These twice-burnt areas had a 17 year recovery period which is a relatively short interval between stand-replacing fires for this species. *Eucalyptus delegatensis* saplings in these areas had not yet reached full maturity leaving these stands vulnerable to poor seedling recruitment or potential recruitment failure. The time to re-establish a canopy seed bank makes *Eucalyptus delegatensis* vulnerable to local extinction if a second fire occurs before adequate canopy seed is produced. Although the primary juvenile period of *Eucalyptus delegatensis* has generally been thought to be in the order of 20 years, recent evidence from stands in the Australian Capital Territory has shown that the species can produce viable seed in as little as 6 years post-fire (Doherty *et al.* 2017a). How widespread this phenomenon is across the full range of stands on mainland Australia is not yet documented.

Table 1. Regeneration status of stands of Alpine Ash in Kosciuszko National Park after the 2020 fires.

Regeneration Status	Pre-2003	12 months post-2003		12 months post-2020	
		Area (ha)	% of AA stands in KNP	Area (ha)	% of AA stands in KNP
Old Mature	77,050 ha	48,425	63	29,348	38
Sapling Maturing	Small area	0	0.0	38,393	50
Seedling Immature	Small area	28,625	37	9,309	12

Background to the Establishment of the Alpine Ash Monitoring Study in Kosciuszko National Park

In late October 2006, an unplanned fire started adjacent to Tooma Dam in KNP and burned 4000 ha of forest, all of which had also burned in 2003. Within the burn perimeter, *Eucalyptus delegatensis* stands occupied approximately 1000 ha. Because these stands had burnt in rapid succession in 2003 and 2006, there was an impetus to develop a monitoring programme to measure *Eucalyptus delegatensis* recovery specifically in the Tooma area and more broadly across KNP.

The life history of *Eucalyptus delegatensis* means that monitoring the species status and post-fire recovery in KNP must use fire history to ensure that plots are representative of the range of growth stages present in the park population. Monitoring of the *Eucalyptus delegatensis* plots has a short-term and a long-term aim: firstly to examine the impacts of and recovery from the 2003 and 2006 fire events and secondly, to monitor the impacts of climate change, focussing on any increases in fire frequency which have the potential to cause significant and lasting impacts on fire sensitive plant communities. The recent fires of 2019/2020 added further impetus to monitor *Eucalyptus delegatensis* stands for the impacts of recurrent fire. In this paper we report on the initial results of post-fire monitoring of *Eucalyptus delegatensis* germination and establishment in KNP after the 2003 and 2020 fires and outline areas for further research and monitoring.

Methods

Initial Stratification and Plot Selection

After the 2003 fires, a representative floristic and structural survey of *Eucalyptus delegatensis* dominated stands in KNP was developed using ArcGIS V9 (ESRI 2004) to stratify potential sample plot locations by fire history, elevation, and aspect. The primary stratification unit was fire severity mapping that was prepared after the 2003 fires (Barrett 2006). Work conducted after a fire near Tooma Dam in 2006 showed that the fire severity mapping categories of 'medium', 'high' and 'very high' were correlated with crown loss, with intact crowns corresponding to 'very low' and 'low' severities. This allowed the entire area of the 2003 burn to be used in the stratification.

For all of KNP, the categories used were: 'not burnt', 'burnt but with an intact crown', or 'crown loss'. As the distribution of *Eucalyptus delegatensis* in KNP is strongly determined by elevation, a 25 m grid cell digital elevation model (DEM) was used to sub-divide the environment in which *Eucalyptus delegatensis* grows. Total annual radiation was also used in the stratification as radiation is a useful predictor of growth rate, with *Eucalyptus delegatensis* seed collected from plots of high insolation growing faster when planted in a range of different environments than seed from areas of low insolation (Garnier-Gere & Ades 2001). ArcGIS V9 was used to model radiation over the whole of KNP, in units of watt-hours/square metre. All layers were clipped to the boundaries of *Eucalyptus delegatensis* and associated canopy dominants using existing aerial photograph interpretation produced as part of the Southern Comprehensive Regional Assessment (Gellie 2005). The 'Combine' function was used to allocate grid cells to each combination of elevation, radiation and fire severity. Two classes of elevation and two classes of annual radiation were used in conjunction with the fire severity mapping to stratify the plot locations. An ArcGIS extension was used to randomly allocate eight replicate plots within each stratum. The potential plots were examined on the GIS, and repositioned where necessary to ensure that they were at least 50 metres from the boundary of the stratum.

Plot Sampling Regime

Initial sampling of 75 *Eucalyptus delegatensis* plots was undertaken from 2008-2010. After this initial sampling, an Australian Alps Liaison Committee (AALC) funded project was established in 2013 to re-monitor *Eucalyptus delegatensis* stands burnt at high severity in 2003 and those plots which re-burnt in 2006. New plots were also established to ensure that the full geographic range of *Eucalyptus delegatensis* within KNP was sampled. During this survey an additional 12 plots were established in *Eucalyptus delegatensis* stands. In total, between 2008 and 2013, 87 monitoring plots across the range of *Eucalyptus delegatensis* dominated vegetation were established and sampled by the NSW Department of Planning, Industry and Environment (DPIE) and Southern Branch of the NSW National Parks and Wildlife Service (NPWS). Plot size was consistent across all the plots sampled (see plot sampling methods below).

In April 2020 prior to the 2021 re-sampling and before any *Eucalyptus delegatensis* seedling recruitment had occurred, traverses were undertaken in selected areas that were burnt at high severity in both 2003 and in 2020 to ascertain the degree of capsule development on saplings that germinated after 2003. In total, 816 trees were examined in ten traverses in stands on Alpine Creek fire trail, Sawyers Hill, the Link Road, around Cabramurra, Snow Ridge Road, and Tooma Rd west of Round Mountain. All trees on the path of the traverse were assessed for capsule development. The time spent in each stand was proportional to stand size and varied from thirty minutes to two hours.

In 2021, 35 of the 37 *Eucalyptus delegatensis* plots identified as having been burnt in January 2020 were re-sampled. These plots were allocated into two categories based on the severity of the 2020 fires:

a) Crown Loss 2020 (High Severity): 21 plots, of which 12 were burnt at high severity in 2003 and nine were burnt at low severity in 2003,

b) Crown Intact 2020 (Low Severity): 14 plots, of which four were burnt at high severity in 2003 and 10 were burnt at low severity in 2003.

This paper discusses only those 21 plots where crown loss occurred after high severity fire in 2020.

Plot Sampling Methods

For the establishment of the initial plots up until 2013 a centre post was established at each plot and location was recorded to +/- 5m with a handheld GPS, so plots could be relocated. Portrait and landscape photographs were taken from the rear of each plot looking downslope. At each plot, a square plot of 20m x 20m around the centre post was used to record vegetation structure and vascular plant cover abundance, using a 0-6 modified Braun-Blanquet scale (Poore 1955).

For tree measurements, a 17.8 m radial plot (0.1 ha) from the centre of the plot was used in which seedlings, saplings (<2 m) and the diameter at breast height (DBH; 1.3 m above ground) of trees greater than two metres in height were recorded. Tree measurements were undertaken by each quarter of the radial plot for ease of counting.

For plot resampling in 2021, the same approach was used and ground truthing of the mapped fire severity class was also undertaken to confirm canopy loss. In addition, seedling counts were undertaken in each quarter of the radial plot, added together, and rounded to the nearest five seedlings for each plot. In this paper, we only report on the results of the seedling count analysis from 2021.

Analysis

Seedling counts were log transformed to bring the data closer to a normal distribution. To test for significant differences between the treatments, a two-sample t-test assuming unequal variances was undertaken on the transformed data in EXCEL. Model fitting was also undertaken on the transformed data, using a negative binomial GLM, with a log link function, in R (R Core Team 2020).

Stem data collected in 2013 also provided a basis to compare seedling germination post-2020 with pre-fire stand characteristics. For those plots burnt at high severity in both 2003 and in 2020, seedling counts were compared to sapling counts (DBH < 10cm). For those plots burnt at low severity in 2003 and high severity in 2020, seedling counts were compared to stem counts (DBH > 10cm) and mean DBH.

Results

Capsule Counts

Of the 816 standing dead *Eucalyptus delegatensis* trees which were assessed six months after the 2020 fires, 63.4% had no capsules (Table 2).

In these instances, it is unlikely that capsules had been consumed during the 2020 fire as overall, trees that had been burned with sufficiently high severity to consume all the branchlets still possessed capsules. Of the trees that did have capsules, 22.9% had >10 capsules per tree.

This was compared to data collected in 2014 on 24 plots which were burned at high severity in 2003. At 11 years post-fire, the percentage of regenerated saplings with fruit in 15 of these plots ranged from 3.4% to 38.7%. A further nine plots surveyed at the same time had much lower numbers of saplings. Five of these plots had no fruiting saplings 11 years post-fire, the other four plots had between one and nine fruiting individuals.

Table 2. Capsule counts on Alpine Ash saplings which germinated after the 2003 fires. Counts were undertaken in April and May 2020.

Number of Capsules per Tree	Number of Trees	%
>100	36	4
10-99	143	18
<10	120	15
None	517	63
TOTAL	816	100

The DBH was measured for 108 fruiting and 244 non-fruiting saplings during the survey work, but there was no significant difference in mean diameter between fruiting (mean DBH 6.1 cm) and non-fruiting (mean DBH 5.3 cm) saplings (t=1.52; df=172; P=0.12).

Alpine Ash Seedlings

For the 12 plots burnt at high severity in both 2003 and 2020, the seedling count ranged from 8 to 1835 seedlings per 0.1 ha, with a mean count of 646 per 0.1 ha (Figure 3). Plot 26 above Tumut Pondage and Plot 69 at Sawyers Hill had very low seedling counts of eight and 26 seedlings per 0.1 ha respectively. For the nine plots burnt at low severity in 2003 and high severity in 2020, the seedling count ranged from 385 to 6930 seedlings per 0.1 ha, with a mean count of 2305 per 0.1 ha (Figure 3).

There was a large apparent difference in means between the treatments, but a high variance associated with the data The seedlings numbers in the Low/High treatment in particular show a wide range of variation across the plots and there is a large degree of overlap in the number of seedlings with the High/High treatment. After log transformation, results from the t-test indicated that there was a weak but significant tendency (t=2.49; df=19; P=<0.05) for higher germination rates in the Low/High severity combination.

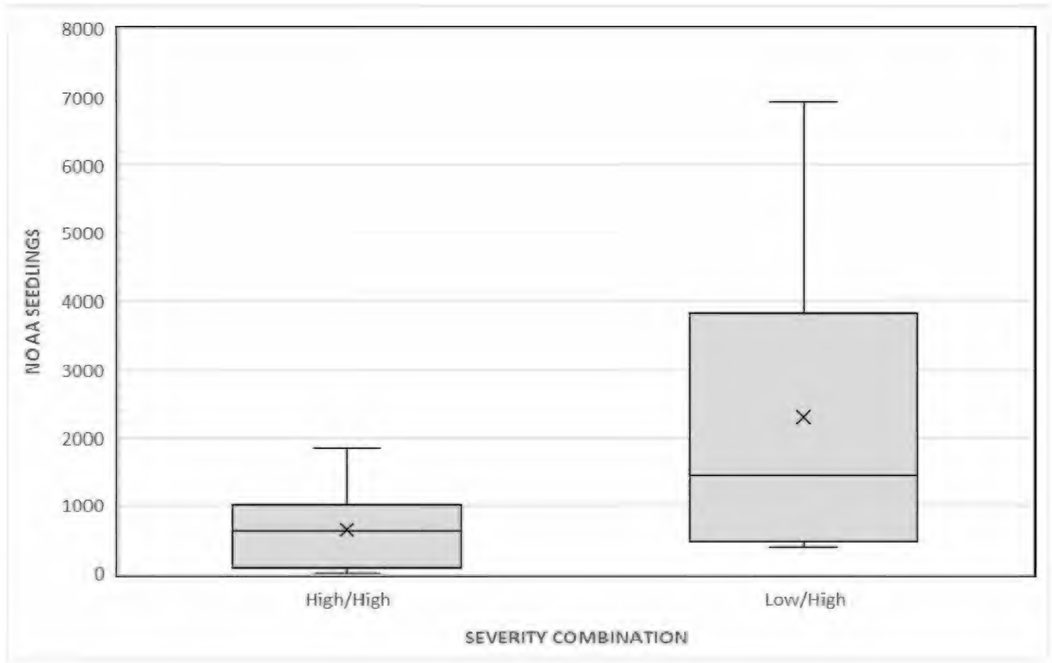

Figure 3. Box and Whiskers plot showing Alpine Ash seedling count raw data mean (cross within box), median (horizontal line within box), 25th and 75th-percentile (box boundaries) and highest and lowest values (whiskers) between areas burnt at high severity in 2003 and in 2020 (LHS) and areas burnt at low severity in 2003 and high severity in 2020 (RHS).

The application of a negative binomial GLM to the data showed a highly significant tendency (P=<0.01) for higher germination rates in the Low/High severity combination:

The limited data from capsule counts shows a moderate linear correlation with seedling counts (R^2=0.73; P<0.05), but with a high degree of variability from plot to plot (Figure 4). The data suggest that by the time 25% of individuals in a stand are fruiting, the post-fire seedling response after high severity fire is likely to be adequate for stand re-stocking.

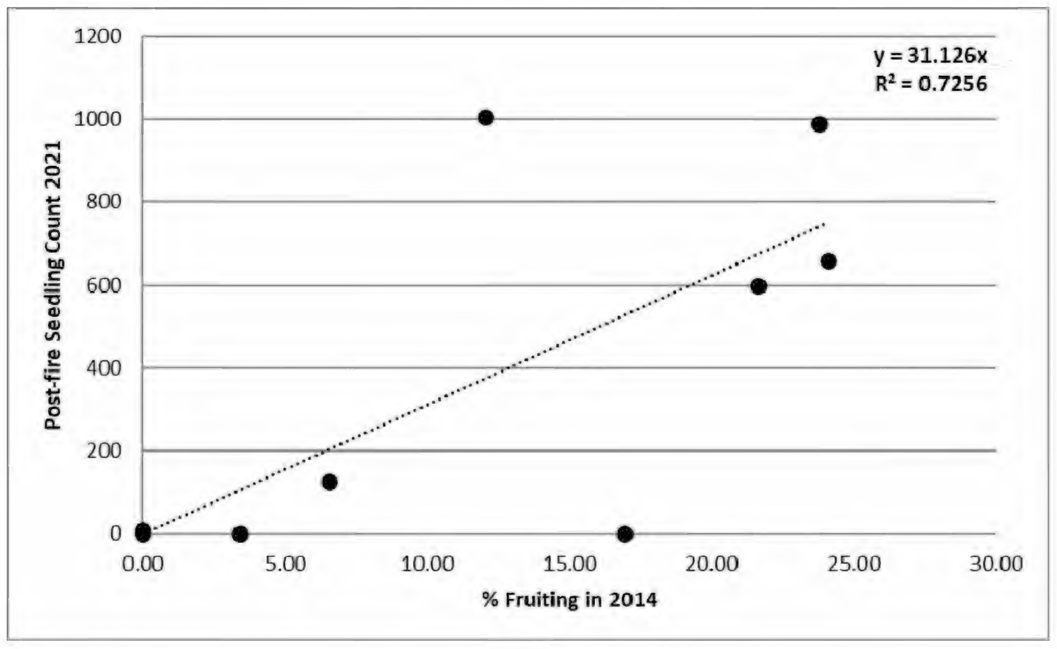

Figure 4. Relationship between fruiting on Alpine Ash saplings that germinated post-2003 and counted in 2014, to Alpine Ash seedling counts post-2020, for 9 of 12 plots burnt at high severity in 2003 and 2020.

The relationship between seedling emergence and elevation varied between those plots burnt twice at high severity and those only burnt at high severity in 2020. In the former, there was a moderately strong polynomial relationship (R^2=0.61; P<0.05) with a peak of seedlings at middle elevations (1200-1300 m) and drop in numbers at both lower and higher elevations (Figure 5). In the latter case, there is a similar but weak polynomial relationship (R^2=0.28; P=0.46) with a general increase in seedling numbers with increasing elevation (Figure 6).

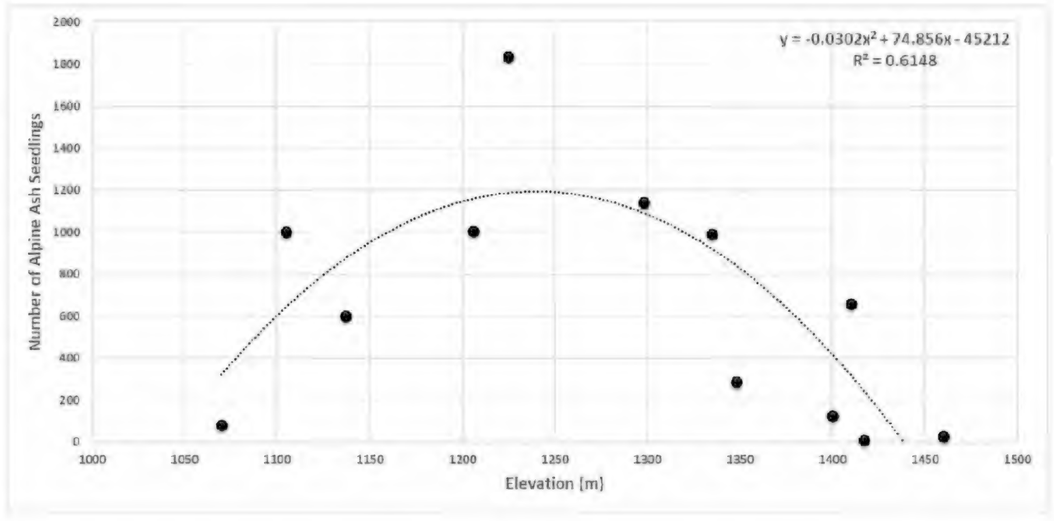

Figure 5. Relationship between elevation and numbers of Alpine Ash seedlings for 12 plots burnt at high severity in 2003 and high severity in 2020.

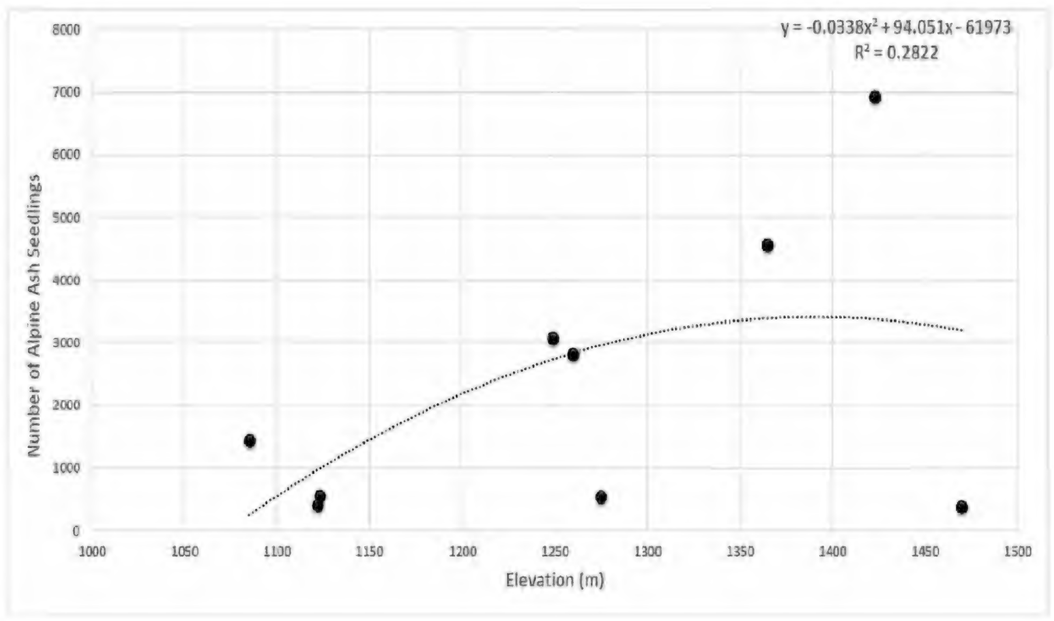

Figure 6. Relationship between elevation and numbers of Alpine Ash seedlings for 9 plots burnt at low severity in 2003 and high severity in 2020.

Using 2013 data, the relationship between elevation and stem counts 10 years after the 2003 fires was used for comparison. For plots burnt at high severity in 2003, the resulting pattern of numbers of saplings showed a weak polynomial relationship (R^2=0.12; P=0.72) with a peak in the number of saplings found in stands of *Eucalyptus delegatensis* at intermediate elevations (Figure 7).

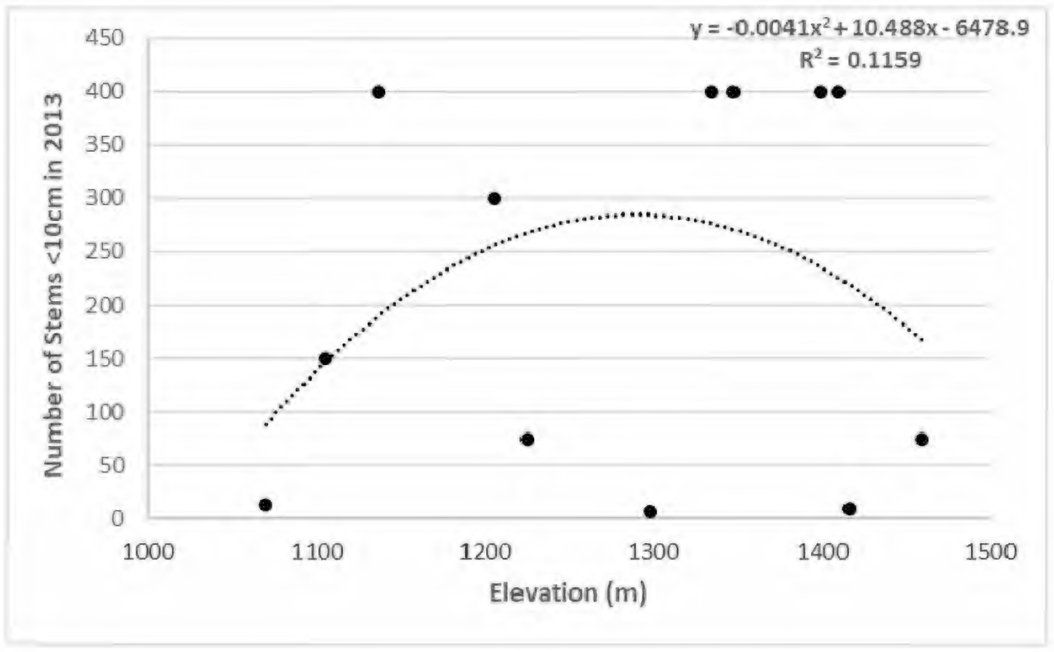

Figure 7. Relationship between elevation and number of Alpine Ash stems found in 12 plots burnt at high severity in 2003, as measured in 2013.

For plots burnt at low severity in 2003, the resulting pattern of numbers of large stems in 2013 showed a weak polynomial relationship (R^2=0.30; P=0.44) with a peak in the number of stems of trees at intermediate elevations (Figure 8).

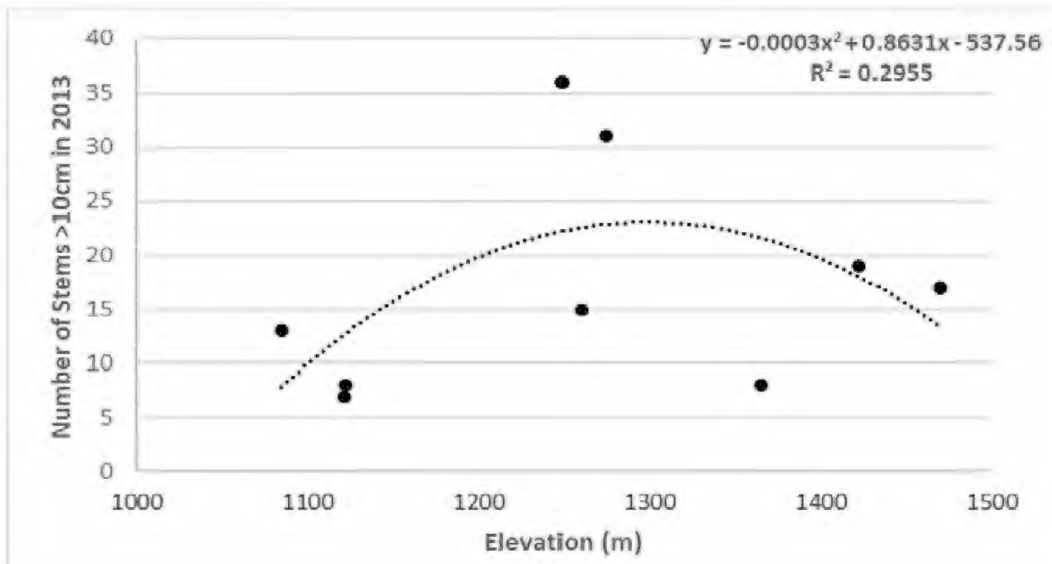

Figure 8. Relationship between elevation and number of Alpine Ash stems found in 9 plots burnt at low severity in 2003, as measured in 2013.

These data from the 2013 and the 2021 samples suggest that there is an optimum elevation for recruitment of *Eucalyptus delegatensis* between approximately 1200 m and 1300 m, in the middle of the altitudinal range of the species in KNP.

There was only a weak relationship (R^2=0.16; P=0.61) between number of sapling stems <10cm DBH and seedling recruitment in plots burnt at high severity in both 2003 and 2020 (Figure 9). The relationship suggests a peak of seedlings at intermediate numbers of saplings (approximately 200).

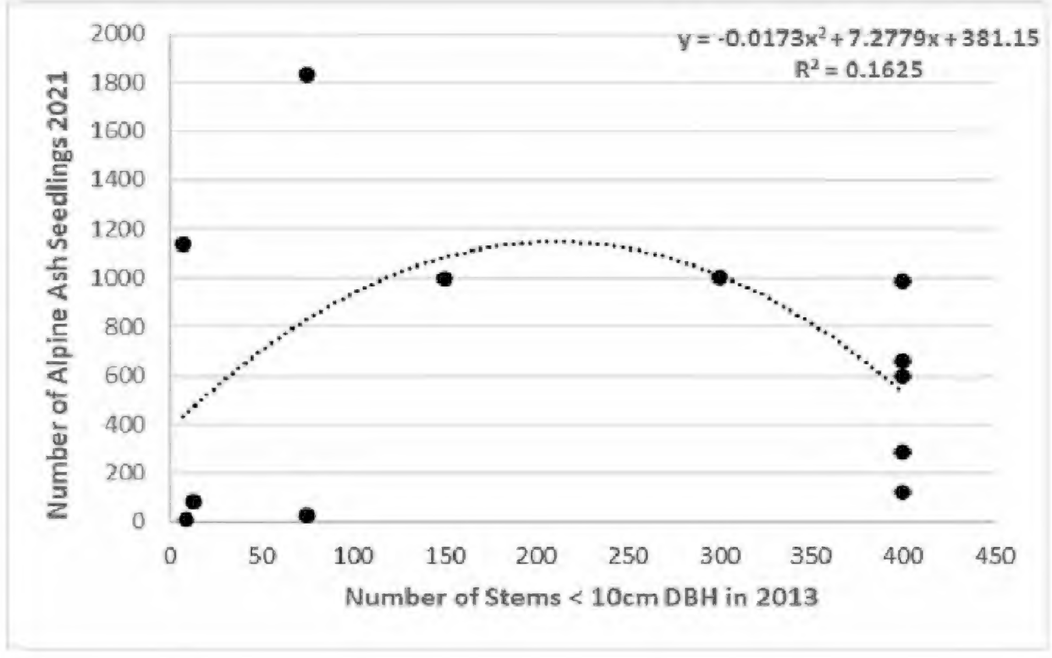

Figure 9. Number of stems of Alpine Ash <10cm DBH in 2013 vs Alpine Ash seedling count in 2021 for 12 plots burnt at high severity in 2003 and high severity in 2020.

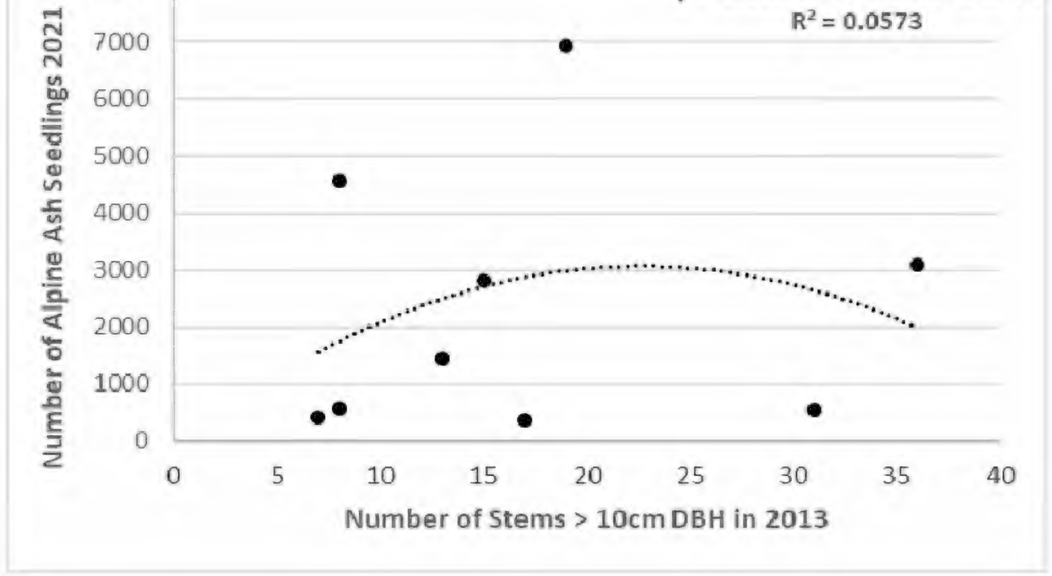

Figure 10. Number of Alpine Ash stems >10cm DBH in 2013 vs Alpine Ash seedling count in 2021 for 9 plots burnt at low severity in 2003 and high severity in 2020.

Similarly for those plots burnt at low severity in 2003 and high severity in 2020, there was a very weak polynomial relationship (R^2=0.06; P=0.88) between number of stems >10cm DBH (Figure 10) and post-fire seedlings with a similar mid-range peak of number of stems to number of seedlings post-fire.

All Eucalypt Seedlings

As well as a detailed count of *Eucalyptus delegatensis* seedlings on the plots sampled, seedlings of other co-occurring eucalypts were counted. The other common co-occurring eucalypts that grow with *Eucalyptus delegatensis* are *Eucalyptus dalrympleana* subsp. *dalrympleana*, *Eucalyptus pauciflora*, *Eucalyptus robertsonii* subsp. *robertsonii* and *Eucalyptus viminalis*. All these species will resprout after high severity fire and given the re-sprouting ability of these species, they are not dependent on seedling recruitment after fire in the short term (Bryant 1971; Noble 1984).

The overwhelming majority of seedlings observed on plots burnt at high severity in 2020 were *Eucalyptus delegatensis* (Figure 11). There is a slightly higher percentage of *Eucalyptus dalrympleana* subsp. *dalrympleana* seedlings in the plots burnt twice at high severity, but this difference is still only a very small percentage of the total number of seedlings counted.

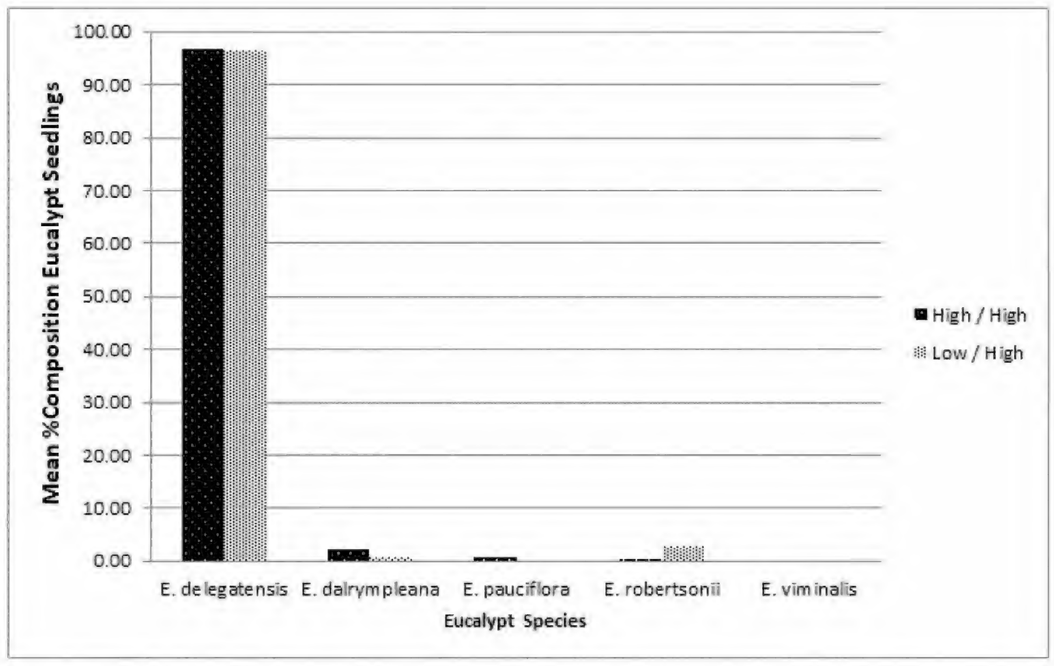

Figure 11. Percentage composition of eucalypt seedlings in plots burnt at high severity in 2020. High/High plots burnt at high severity in 2003 and in 2020; Low/High plots burnt at low severity in 2003 and at high severity in 2020.

Discussion

Capsules

Despite the relatively short 17-year interval between high severity fires in 2003 and 2020 in KNP, successful seedling recruitment of *Eucalyptus delegatensis* has occurred on all plots burnt at high severity in 2020. The relatively low numbers of fruiting capsules in many stands burnt at high severity in 2003 is not surprising, but the high variability between plots with the same fire history means that some plots have much higher capsule counts. The resulting trend that hundreds of seedlings are likely to establish when more than 25% of trees in a stand have fruited was partially a time-since-fire

effect, but also a function of differential growth rates post-fire between plots.

In *Eucalyptus delegatensis*, flowering and fruiting can begin from at least six years of age, so a small but growing canopy seed bank was accumulating for 11 years between the two high severity fire events. Wright and Robertson (2014) found evidence of *Eucalyptus delegatensis* stands in KNP producing fruit within 10 years of the 2003 fires and it has been established that *Eucalyptus delegatensis* seedlings can produce fruit and importantly, viable seed, from at least six years after stand replacing fires in the northern parts of the Australian Alps (Doherty *et al.* 2017a). Given that the time from bud initiation to fruit development can take over three years (Cremer *et al.* 1984), some individuals may be beginning to develop buds as early as three to four years after germination. Field observations to date suggest that suppressed individuals in *Eucalyptus delegatensis* stands are more likely to flower and fruit earlier than fast growing individuals. This contrasts with *Eucalyptus regnans* (Mountain Ash) in Victoria, where rapidly growing individuals are more likely to produce seed (von Takach Dukai *et al.* 2018). Early flowering and fruiting has also been recorded in other fire-killed ash group eucalypts in NSW including *Eucalyptus oreades* (Blue Mountains Ash) (Glasby *et al.* 1988) and *Eucalyptus fraxinoides* (White Ash) (Newbery *et al.* 2022).

Seedlings

Although *Eucalyptus delegatensis* seedling numbers at plots which burnt *only* at high severity in 2020 are significantly higher than those at plots burnt at high severity in *both* 2003 and 2020, this initial difference may not necessarily affect stand structure in the medium to long term. Larger numbers of *Eucalyptus delegatensis* seedlings emerging post-fire increases the probability of at least some individuals persisting and ultimately attaining reproductive maturity. However, there is naturally a very high attrition rate of seedlings and saplings until a mature forest structure is attained (Forestry Tasmania 2010). Most of the germinating seedlings will ultimately die during the time it takes to establish a mature tall open forest structure. Conceptually, a stand could reach a mature structural state after self-thinning from a very large initial number of seedlings developing into saplings over many decades, or else a stand could reach a similar structure through a less intensely competitive process starting with significantly fewer seedlings. At present, there is insufficient information to evaluate the relative time frames of either scenario, particularly under short-interval fire. However, at this time post-fire in the areas surveyed, there is adequate seedling recruitment to naturally re-stock stands burnt at high severity in 2020, notwithstanding any future high severity fire events that may occur.

For stands burnt twice at high severity the mid-elevation peaks in seedlings post-fire - with low numbers at the low and high ends of the altitudinal range of the species - suggests that macro-climatic and micro-climatic factors e.g. length of snow cover, severity of frost and solar radiation may play a part in keeping seedling numbers lower post-fire at the extremes of the species range (Battaglia and Reid 1993; Bowman and Kirkpatrick 1986b; Florentine *et al.* 2008). These post-fire environmental factors and the interaction with fire frequency and fire severity likely bound the realised niche of *Eucalyptus delegatensis* in the Australian Alps (Gale and Cary 2021; Singh *et al.* 2021). But whether there are thousands or hundreds of seedlings in a stand, they are equally susceptible to short-interval fires.

High severity fire not only provides the necessary conditions for successful *Eucalyptus delegatensis* seedling regeneration, but also for the regeneration of other co-occurring eucalypts. Over evolutionary time, the interaction of fire regimes with climate, micro-climate and substrate has produced both pure and mixed stands of *Eucalyptus delegatensis* in KNP. In mixed stands, there are at least some individuals of *Eucalyptus dalrympleana* subsp. *dalrympleana*, *Eucalyptus robertsonii* subsp. *robertsonii* or *Eucalyptus pauciflora* present. The mixing of other eucalypt species within *Eucalyptus delegatensis* dominated stands, as well as the proximity of *Eucalyptus delegatensis* stands to other montane forest types dominated by other eucalypt species, would suggest that the composition of stand dominants could change after high severity fire with seedling recruitment from a range of eucalypt species. However, evidence to date in the northern extent of the range of *Eucalyptus delegatensis* suggest that this is not happening in the short term, with stand boundaries stable after high severity fire (Vivian *et al.* 2008).

Because all eucalypt species carry a canopy seed bank there are opportunities for all species present to regenerate from seed post-fire (Hillis and Brown 1984; House 1997; Pryor 1976). The advantage that other co-occurring eucalypt species have over *Eucalyptus delegatensis* is that they are able to resprout from epicormic shoots or basally, enabling a greater ability to persist under recurrent fire irrespective of seedling recruitment (Gill 1975; 1981). It is therefore expected that changing fire frequencies and severities under climate change will put selective pressure on stand composition and structure. The resprouting ability of eucalypt species other than *Eucalyptus delegatensis* may lead to a transition in composition and structure from fire-killed *Eucalyptus delegatensis* stands to more fire tolerant mixed eucalypt stands, but whether this happens naturally or needs to be facilitated is an open question (Colloff *et al.* 2016; Doherty *et al.* 2017b). Although fire-killed *Eucalyptus delegatensis* stands with little or no natural regeneration have been successfully re-seeded in some parts of the Victorian alps (Bassett *et al.* 2015), such stands remain vulnerable to repeated fire despite a large financial re-investment in re-seeding. Additionally, given the potential for differential performance of the species across its elevational range based on existing genotypic variation, we suggest a cautionary approach is warranted to broad area re-seeding until more is known about the mixing of *Eucalyptus delegatensis* genotypes.

Observations of current sapling and seedling regeneration show that most of the current extent of *Eucalyptus delegatensis* tall open forest in KNP is either mature or else is still capable of growing into mature tall open forest contingent on the next high severity fire event. However, with very frequent high severity fire, *Eucalyptus delegatensis* stands could become open with few stems, potentially moving in a trajectory toward shrubland or grassland. Such transitions have to date occurred in small areas, as for example in some stands of *Eucalyptus regnans* in Victoria after the 1939 fires (Ashton 2000; Costermans 2010) but there is concern that much larger areas of montane forest will be susceptible to rapid transitions to non-forest vegetation under a future of short-interval high severity fires (Bowd *et al.* 2023). Alternatively, in mixed stands where there are also some resprouting eucalypt species present, a trajectory toward increasing dominance by resprouting species could occur, involving a transition to a different forest type rather than a transition from forest to shrubland or grassland (Doherty *et al.* 2017b). Both possibilities could occur in KNP.

There are signs in some plots of what a transition may look like if fire frequency increases. An example is Plot 69 (Figure 12), which was burnt in 1939 and had successful recruitment and regeneration after the 2003 fire event, but which has had poor recruitment after the 2020 fires, with only 26 seedlings counted in 2021. This plot had a mature but open structure prior to being burnt at high severity in 2003. In more typical mature stands which only burnt at high severity in 2020, the structure showed a greater density of dead stems reflecting the higher stem densities in forest which have as yet not undergone a regime of too frequent high severity fire events (Figure 13).

Figure 12. Alpine Ash stand at Sawyers Hill burnt at high severity in 2003 and at high severity in 2020. Large dead standing trees are those killed in 2003. Layer of dead saplings are those trees which germinated after 2003 and were killed in 2020. Plot 69, elevation 1460 m. (Photo: M. Doherty).

Figure 13. Alpine Ash stand near Cabramurra burnt at low severity in 2003 and at high severity in 2020. Large dead standing trees are those killed in 2020. Plot 25, elevation 1417 m. (Photo: M. Doherty).

In all cases where plots were burnt at high severity, there was a marked flush of shrub, herb and grass re-growth, particularly large tussocks of multiple species of *Poa*. However, there is no evidence that this affected the ability of *Eucalyptus delegatensis* seeds to germinate and establish. The rapid growth rates of *Eucalyptus delegatensis* seedlings in the first twelve months post-fire ensures that they establish well before the re-establishment of shrub, herb, and grass layers (Figures 14 and 15). These understorey layers typically begin to accrue a high biomass and cover approximately two years post-fire and beyond (Doherty and Wright 2004; 2005).

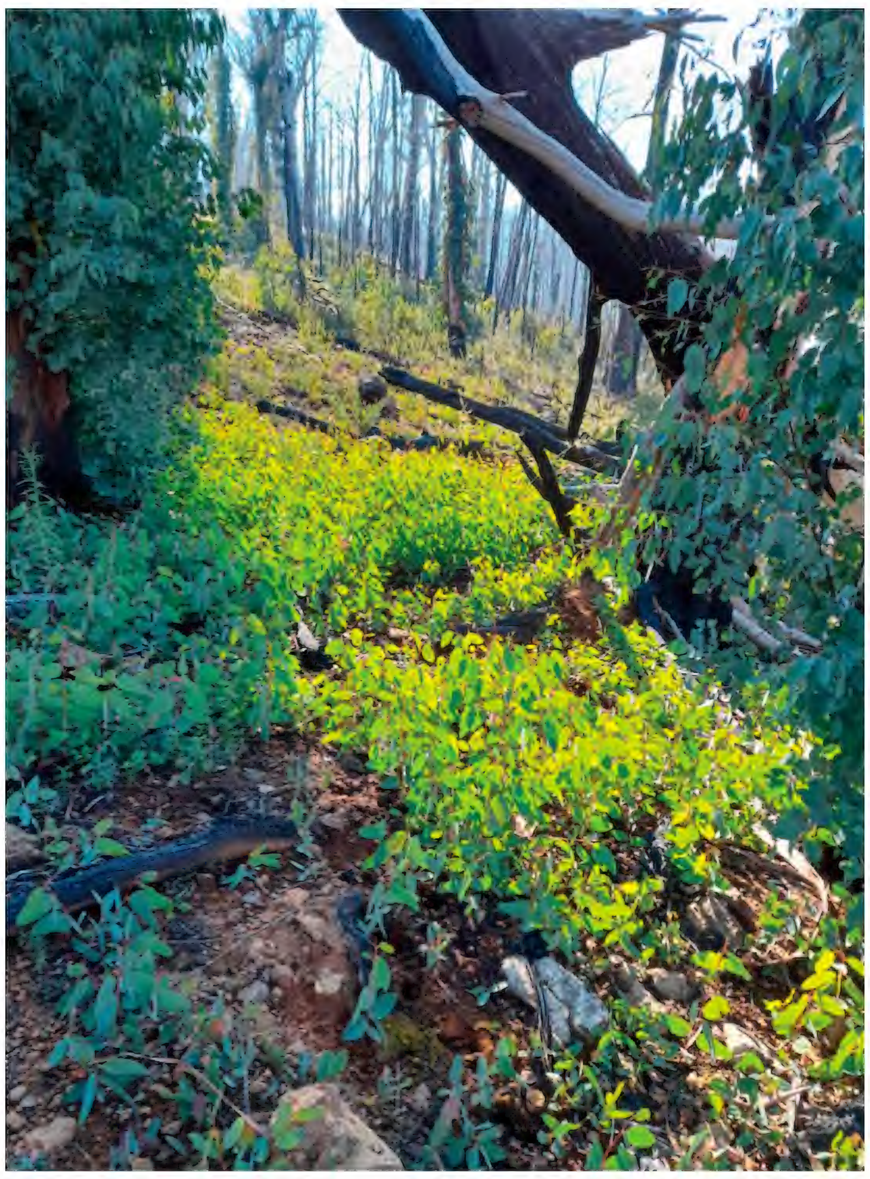

Figure 14. Alpine Ash seedlings north of Yarrangobilly 15 months post-fire. Taller re-sprouting trees on left and right and at rear are Mountain Gum. Near Plot 61, elevation 1225 m, burnt at high severity in 2003 and 2020. (Photo: M. Doherty).

Figure 15. Advanced Alpine Ash seedling 55 cm high northeast of Alpine Hill, 15 months post-fire. Plot 68, elevation 1348 m, burnt at high severity in 2003 and 2020. (Photo: M. Doherty).

In these first two years post-fire, seedling recruitment and regeneration from re-sprouting ensure a rapid establishment of overall plant cover. After this initial flush, plant growth rates slow as competition for light, water and nutrients becomes more intense.

Fire extent and frequency are expected to increase in the medium to long term in southeastern Australia, putting further pressure on fire-killed ash stands (Bowman *et al.* 2016; McColl-Gausden *et al.* 2021). Our dataset makes a significant contribution to understanding recruitment and survival in eucalypts post-fire in the Australian Alpine Bioregion. Monitoring the growth and survival of the post-2020 cohort of *Eucalyptus delegatensis* seedlings will be critical to inform appropriate fire management and it will be important to document the time to first flowering and fruiting of individuals in fire-affected stands and to track the proportion of saplings reaching reproductive maturity over time, to gauge stand vulnerability to further high severity fires over coming decades.

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New records of plant pathogen *Phytophthora* species in Kosciuszko National Park, southeastern Australia

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Abstract: *Phytophthora* species in Australia are microscopic soil- and water-borne pathogens, which have caused great economic and environmental damage. Recent surveys of Kosciuszko National Park have detected 15 *Phytophthora* species through baiting and a further 10 species from high throughput DNA sequencing. Some of these species may be native pathogens. The hosts in the park of only two of the 15 baited species are known: *Pimelea bracteata* is a host of *Phytophthora gregata* and *Nematolepis ovatifolia* is a host of *Phytophthora cambivora*. Of the species recorded, *Phytophthora cinnamomi*, *Phytophthora gregata* and *Phytophthora multivora* are likely to be the most destructive of environmental values.

Very little of the park is currently suitable for *Phytophthora cinnamomi* but the area of suitable habitat is likely to increase in the coming decades as mean temperature increases; a few subalpine plant species are already known to be badly affected by it following infection. Preventing the dispersal of *Phytophthora* species in Kosciuszko National Park will be difficult because of the unfettered movement of vehicles, mountain bikes, horse riders and walkers through much of the park, and the increasing numbers of large feral animals present. The identification of protectable at-risk plant populations will be a priority for land managers in the future.

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Introduction

The Phylum Oomycota is a group of micro-organisms collectively called oomycetes and commonly known as water moulds. Many oomycetes are pathogenic and are responsible for common plant diseases such as damping off (*Pythium* species), downy mildew (e.g. *Peronospora* species), and dieback, a term encompassing a range of symptoms caused by *Phytophthora* species. *Phytophthora* species are amongst the world's most destructive plant pathogens, causing catastrophic economic damage (e.g. *P. infestans* to potatoes, contributing to the Irish famine of the 19th Century) and environmental degradation (e.g. *P. cinnamomi* in heathlands and forests of southern Australia and *P. ramorum* in oak forests of North America) (Kroon et al. 2012). All species of *Phytophthora* recorded in Australia are soil- or water-borne, colonising roots and lower stems.

Burgess et al. (2021) compiled a list of 99 *Phytophthora* species in Australia from almost 8000 records, an increase from the 27 species known in the year 2000, following extensive surveys and improvements in molecular diagnostic techniques. Some of the species recorded in Australia are likely to be native, although the exact number is unknown because surveys have not been as extensive overseas (i.e. the global distribution is often unknown and trade networks have undoubtedly dispersed geographically isolated species).

Kosciuszko National Park (KNP) is the largest reserve for nature conservation in New South Wales (NSW) and is characterised by its steep environmental gradients, which produce a great diversity of habitats (from rain-shadow *Callitris* woodlands, to rainforest and alpine communities); the park supports almost one quarter of the New South Wales (NSW) flora and many endemics (Doherty et al. 2015). Until 2014, to our knowledge, there had been no surveys for *Phytophthora* species in the park. Before that time, the subject of most pathogen surveys in Australia was *P. cinnamomi*, and cold, mountainous areas were thought to be unsuitable for its activity (e.g. Podger et al. 1990). Since then, surveys associated with the Mountain Invasion Research Network (MIREN; Burgess et al. 2017, Khaliq et al. 2021) and those responding to observations of poor plant health have added greatly to knowledge about *Phytophthora* diversity in KNP and high mountain areas of Australia in general.

Phytophthora species may be detected in soils and plant material in two ways. The first involves baiting of soil and plants in a wet slurry using attractants, such as agar or susceptible plant material (e.g. lupins), to attract motile *Phytophthora* zoospores. If *Phytophthora* species are taken up by the baits, they are then cultured and identified by morphology and / or DNA sequencing. Gene sequence databases may be used to identify entities to species level. At times, a species name cannot be given to the detected entity either because it has not been detected before (and may be a new species) or the gene sequence was inadequate for matching. Importantly, species identified in this way were alive when sampled, either as vegetative material or spores (short-lived zoospores or resting spores known as a chlamydospores). High Throughput Sequencing (HTS) may also be used to identify *Phytophthora* species in soil and plant samples. This locates fragments of DNA and matches them against known sequences in the database. This technique may identify both live organisms and fragments of DNA from organisms that were once present but have not persisted at a site.

In this paper, we review the results of surveys for *Phytophthora* in KNP since 2015, some of which are unpublished, and present the results of a repeat survey in 2022 of sites sampled in 2017. We then focus on the current and future modelled occurrence of *P. cinnamomi* in the park, and provide management recommendations for the protection of flora at risk from *Phytophthora* pathogens in KNP.

Previous studies of *Phytophthora* presence in Kosciuszko National Park

The Australian Alps Liaison Committee funded a survey for *Phytophthora* in early 2015 along the Alpine Walking Track in KNP and beyond. Within KNP, 47 soil samples were collected and baited at the Royal Botanic Gardens Sydney. No species of *Phytophthora* were detected in the samples, although two species of the related genus *Pythium* (*Pythium macrosporum* and *Pythium undulatum*) were detected (McDougall and Wright, unpublished data).

A study of *Phytophthora* diversity in Australia (Burgess et al. 2017) tested 17 soils from MIREN plots beside Kosciuszko and Tooma Roads and a selection of other roadsides in KNP for *Phytophthora* presence. Twenty species, species aggregates or taxa of uncertain status were detected using HTS. These species may not persist in the locations where they were detected in the park, and most probably do not. For instance, *Phytophthora cinnamomi* was detected by Burgess et al. (2017) at several sites in the alpine area as high as 2125 m above sea level. However, in a subsequent study, *Phytophthora cinnamomi* was not detected at the same sites using baiting (see Khaliq et al. 2021).

Green (2016) detected *Phytophthora cambivora*, using baiting, in the roots of dying *Nematolepis ovatifolia* shrubs in the alpine zone of KNP near Charlottes Pass.

McDougall et al. (2018) detected *Phytophthora gregata* through baiting in dying *Pimelea bracteata* at two sites in northern KNP. Unlike many *Phytophthora* infections, the pathogen caused distinct lesions in plant collars (in the vicinity of the soil surface) rather than in roots.

Khaliq et al. (2019) isolated six *Phytophthora* species from soil samples using baiting along road and track edges in KNP, including *Phytophthora oreophila*, a newly described species currently only known from the Type location near Merritts Creek in the alpine zone. *Phytophthora cryptogea* was isolated from the summit of Mount Kosciuszko and *Phytophthora gregata* in the vicinity of Perisher ski resort at 1750 m above sea level.

Khaliq et al. (2021) sampled MIREN plots on three roads in KNP in 2017: Kosciuszko Road, Snowy Mountains Highway, and Tooma Road. Two samples were taken at each site:

one on the roadside in highly disturbed vegetation and one 50 – 100 m from the road in natural vegetation. *Phytophthora* detection was done using both baiting and HTS techniques. Nine species were detected using baiting and 16 species were detected using HTS; two species were detected by both baiting and HTS.

Methods for the 2022 survey

A survey in January 2022 sampled soil from the same natural vegetation plots as Khaliq et al. (2021) but only baiting was used for *Phytophthora* detection. Rhizosphere soil samples including fine roots were collected in each plot. Samples were collected from five different places (approximately 10–15 m apart) and placed in a single ziplock bag for each site (up to about 400 g in total). The trowel used for sampling was disinfected using 70% methylated spirits after each sample.

Methods used for *Phytophthora* species detection and identification in 2017 and 2022 were similar in that they used baits to attract any *Phytophthora* species present, cultured the pathogens on selective agar, identified species based on morphological traits and confirmed the identifications by sequencing rDNA. However, in 2017, soil samples were baited at Murdoch University (Western Australia) with juvenile leaves of *Rhododendron* spp., *Quercus ilex*, *Q. suber*, *Pimelea ferruginea*, *Poplar* sp., *Scholtzia involucrata*, and *Hedera helix* (see Khaliq et al. 2021 for a detailed description of the method) while in 2022 they were baited at the Royal Botanic Gardens Sydney using *Lupinus angustifolius* (see McDougall et al. 2018 for a detailed description of the method). While the methods differed between years, the outcomes should similar, given that soils were collected from the same sites.

Results

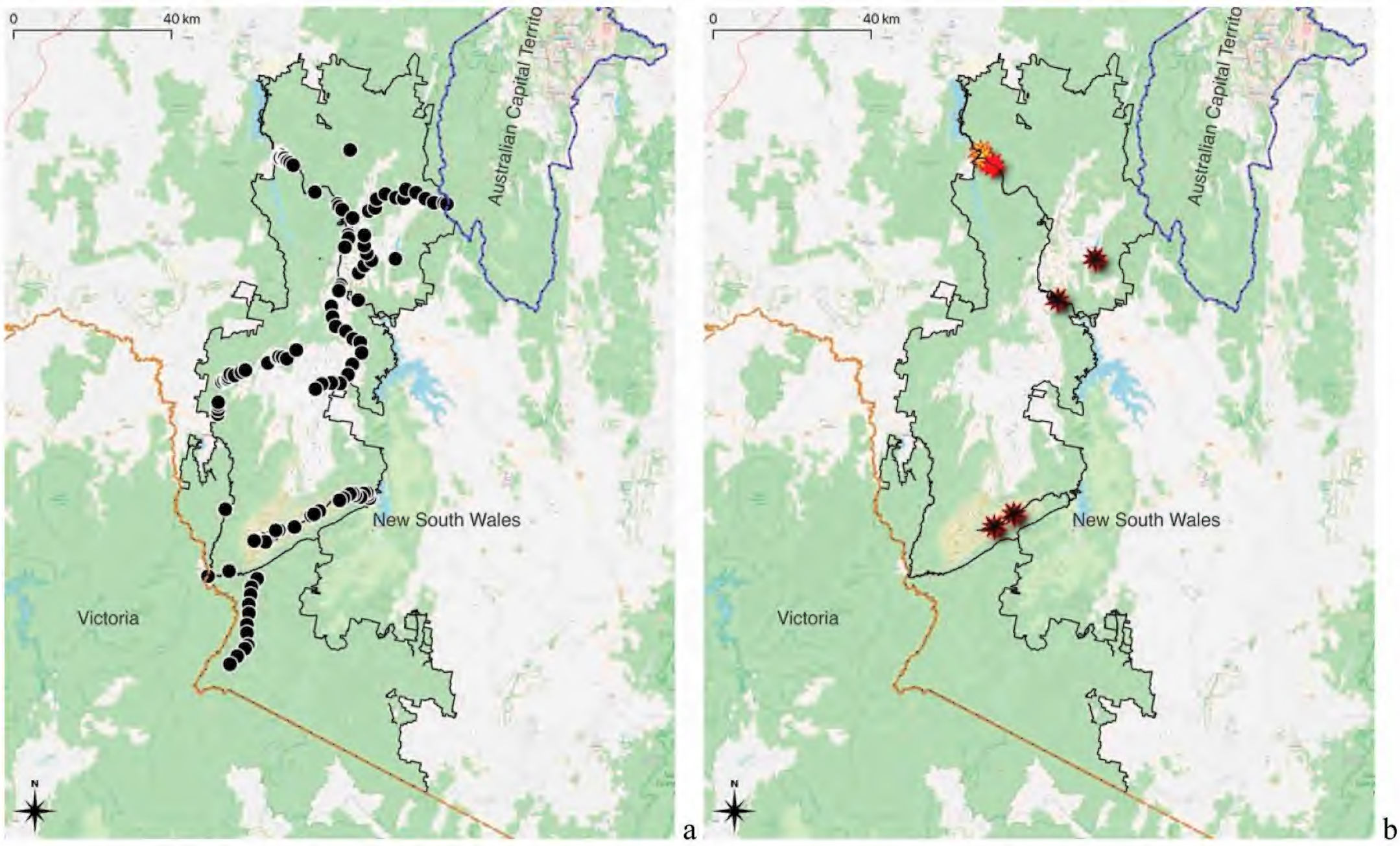
From all surveys, 15 *Phytophthora* species have been detected in Kosciuszko National Park using baiting techniques – these are species that were alive at the time of sampling. A further 10 species have only been detected using HTS and these may not persist in the park (Table 1). For all *Phytophthora* species except *Phytophthora cambivora* and *Phytophthora gregata*, hosts within KNP are unknown and for many *Phytophthora* species, knowledge about hosts extends only to genera present in the park, based on susceptibility testing of species elsewhere (and mostly Western Australia). The optimum temperatures for growth of the species detected in KNP (as per Abad et al. 2023) are between 20 and 30°C but all are capable of growth at 10°C and some as low as 2°C (Table 1).

Table 1. List of *Phytophthora* species detected in Kosciuszko National Park through baiting and High Throughput Sequencing (HTS). Data sources for baiting and HTS detections: 1 - Green (2016); 2 - Burgess et al. (2017); 3 – McDougall et al. (2018); 4 - Khaliq et al. (2019); 5 - Khaliq et al. (2021); 6 – this study. Names not accepted by Burgess et al. (2021) are not included. Minimum – optimum – maximum temperatures for growth are from https://idtools.org/phytophthora/ (Abad et al. 2023). Hosts are listed for each *Phytophthora* species as genera occurring in KNP (based on detections in species not occurring in KNP; McDougall and Liew (unpublished data)), and as species occurring in KNP (in bold; based on glasshouse susceptibility testing by Rigg et al. (2018) and Wan et al. (2020)).

***Phytophthora* Species**	**Baiting**	**HTS**	**Growth temperatures (°C)**	**Australian plant hosts**
Baiting				
P. cactorum	4,5,6	2	4 – 24 - 30	*Banksia, Calytrix, Crowea, Grevillea, Hakea, Pimelea*
P. cambivora	1		2 – 23 - 32	***Epacris petrophila, Grevillea victoriae, Nematolepis ovatifolia, Oxylobium ellipticum, Phebalium squamulosum, Prostanthera cuneata***
P. chlamydospora	5		? – 26.5 – 36.5	None known
P. cinnamomi	6	2	9 – 27 - 30	***Epacris petrophila, Grevillea victoriae* subsp. *nivalis, Nematolepis ovatifolium, Oxylobium ellipticum, Phebalium squamulosum, Podocarpus lawrencei, Prostanthera cuneata***; very large host range, see McDougall (2016)
P. cryptogea	4	2	3 – 24 - 30	*Allocasuarina, Banksia, Bauera, Crowea, Eucalyptus, Grevillea, Hakea, Leucopogon, Lomandra, Patersonia, Pimelea, Podocarpus, Xanthorrhoea*
P. elongata	5,6	2,5	5 – 25 – 32.5	*Banksia, Eucalyptus, Hakea, Leucopogon, Patersonia, Persoonia, Styphelia, Xanthorrhoea*
P. fallax	5	2	2 – 25 - 30	None known
P. gonapodyides	4		3 – 25.5 - 30	None known
P. gondwanensis	5		5 – 27.5 - 32	None known
P. gregata	3,4,5,6	2	7.5 – 25 – 32.5	***Pimelea bracteata,*** *Banksia, Boronia, Correa, Eucalyptus, Pultenaea, Xanthorrhoea*
P. megasperma	6		3 – 18 - 27	*Allocasuarina, Banksia, Boronia, Hakea, Xanthorrhoea*

***Phytophthora* Species**	**Baiting**	**HTS**	**Growth temperatures (°C)**	**Australian plant hosts**
P. multivora	5	2	5 – 25 – 32.5	*Acacia, Allocasuarina, Banksia, Elaeocarpus, Eucalyptus, Grevillea, Hakea, Leucopogon, Mirbelia, Patersonia, Persoonia, Podocarpus, Styphelia, Xanthorrhoea*
P. oreophila	4		4 – 20 – 32.5	None known
P. pseudocryptogea	4,5,6	5	3 – 25 - 35	*Acacia, Banksia, Xanthorrhoea*
P. thermophila	5	2	10 – 33 - 35	*Banksia, Eucalyptus, Persoonia, Xanthorrhoea*
P. acerina		2	6 – 25 - 32	None known
P. arenaria		2,5	10 – 25 – 32.5	*Banksia, Eucalyptus, Hakea, Xanthorrhoea*
P. sp. AUS9B	5	Not known	None known	None known
P. bilorbang	2	4 – 25 – 32.5	None known	None known
P. citricola	2	3 – 24 - 30	None known	None known
P. litoralis	2	10 – 30 - 35	*Banksia, Callitris, Eucalyptus, Xanthorrhoea*	
P. moojotj	2	10 – 27 – 32.5	*Banksia, Eucalyptus*	
P. niederhauserii	2	10 – 30 - 37	*Banksia, Eucalyptus*	
P. nicotianae	2	9 – 24 - 33	*Banksia, Bauera, Billardiera, Boronia, Callistemon, Correa, Crowea, Eucalyptus, Eutaxia, Grevillea, Hakea, Kunzea, Melaleuca, Pimelea, Westringia, Xanthorrhoea*	
P. versiformis	5	4 – 20 - 28	None known	

Many detections of *Phytophthora* species from baiting were on the low elevation sections of the three roads from which most samples came: Snowy Mountains Highway (in the vicinity of Talbingo Mountain), Kosciuszko Road and Tooma Road (Fig. 1). The exceptions to this are *Phytophthora gregata*, which was detected at elevations between 1300 and 1750 m on the eastern side of the park, *Phytophthora cambivora, Phytophthora gonapodyides*, and *Phytophthora oreophila*, which are only known above 1660 m near Kosciuszko Road, *Phytophthora cryptogea*, found on the summit of Mount Kosciuszko (but recorded at lower elevations close to KNP in Bago State Forest and Namadgi National Park), and *Phytophthora cactorum*, found in subalpine grassland near the Snowy Mountains Highway turnoff to Long Plain.



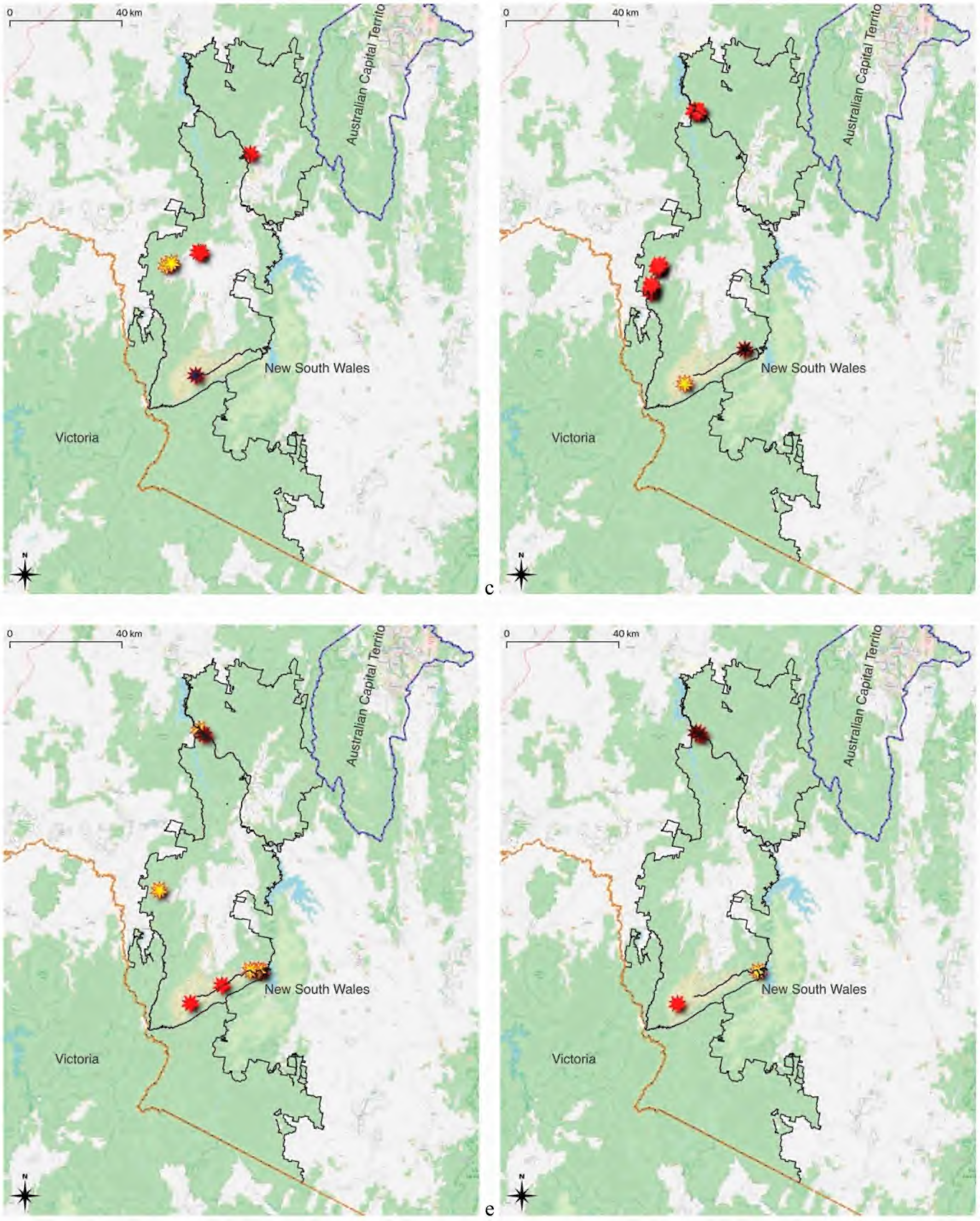


Figure 1. Distribution of *Phytophthora* species detected by baiting in Kosciuszko National Park (black outline) **a.** *Phytophthora* sample sites (black dots); **b.** red = *P. cinnamomi*, black = *P. gregata*, yellow = *P. multivora* (potentially the most destructive *Phytophthora* species in KNP); **c.** red = *P. cactorum*, black = *P. cambivora*, yellow = *P. chlamydospora*; **d.** red = *P. elongata*, black = *P. megasperma*, yellow = *P. oreophila*; **e.** red = *P. gonapodyides,* black = *P. gondwanense*, yellow = *P. pseudocryptogea*; **f.** red = *P. cryptogea*, black = *P. fallax*, yellow = *P. thermophila*.

The single record of *Phytophthora cinnamomi* in KNP was from forest beside the Snowy Mountains Highway near the Black Perry Lookout where the modelled *Phytophthora cinnamomi* habitat suitability is low (0.01 on a scale from 0 to 1; McDougall and Liew 2020) and the modelled mean annual temperature is 10°C. The minimum reported temperature for growth of *P. cinnamomi* is 9°C (Abad et al. 2023). While mean annual air temperature will not capture the annual extremes of temperature, some of which might be unfavourable or lethal to *Phytophthora cinnamomi*, temperature fluctuations in the soil rhizosphere where the pathogen occurs will not be as great as in the air above. The mean annual air temperature is perhaps therefore a reasonable predictor of growth and survival. This suggests that the site near Black Perry Lookout is marginal for *Phytophthora cinnamomi*. If this is the case, only the western edge of KNP and the lower Snowy River valley are currently marginally suitable habitat for *Phytophthora cinnamomi* (Fig. 2a). By 2070, using the CSIRO-Mk3-6-0 climate model (Gordon et al. 2002) and depending on the modelled climate scenario, more of the park will become suitable; under rcp 4.5 (an intermediate climate change scenario), much of the west and north of the park and all of the lower Snowy River area will be suitable (Fig. 2b), while under a rcp 8.5 scenario (a worst case on current projections), all but higher elevations between the Main Range and the Jagungal area will be suitable (Fig. 2c). Rainfall is unlikely to limit growth and survival of *P. cinnamomi* anywhere in the park, now or in the future.

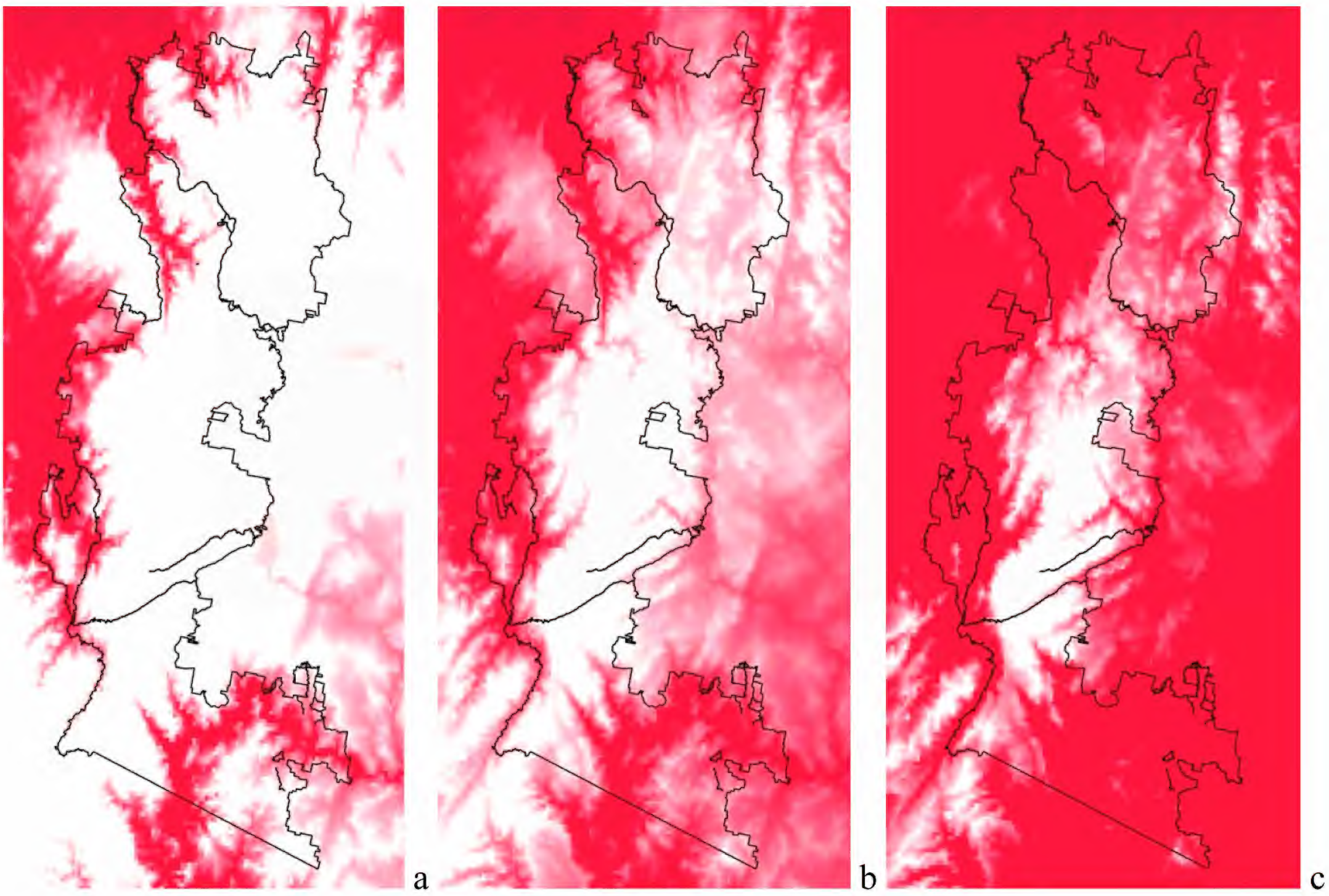


Figure 2. The red shaded area in each panel represents the 10°C isotherm, the mean annual temperature of the site of the only *Phytophthora cinnamomi* record in Kosciuszko National Park (with white areas having a mean annual temperature less than 10°C) – the park boundary is a continuous black line; **a.** current climate, **b.** projected climate in 2070 using the CSIRO-Mk3-6-0 climate model for the rcp 4.5 scenario, **c.** projected climate in 2070 using the CSIRO-Mk3-6-0 climate model for the rcp 8.5 scenario.

Repeat plots

In 2017, *Phytophthora* species were detected through baiting by Khaliq (2021) at 13 sites in natural vegetation (23% of sites sampled) while in 2022 we detected *Phytophthora* species at only 4 sites (7% of sites sampled; Table 2). Six species were identified in samples in 2017, *Phytophthora elongata* being the most frequently detected (7 sites). Four species were detected in 2022, at least two of which were not identified in 2017 – it is likely that *Phytophthora* cf. *cryptogea* detected in 2022 corresponds with *Phytophthora pseudocryptogea* found nearby in 2017. Curiously, none of the species detected in 2022 corresponded with species detected at the same sites in 2017. The detection of *Phytophthora cinnamomi* in 2022 is the first record of this species by baiting in Kosciuszko National Park. Although the soil sample was taken in forest at least 50 m from the road edge, there was considerable disturbance at the road edge from reconstruction of a barrier destroyed by the 2019 / 20 bushfire.

Table 2. *Phytophthora* species recorded in surveys of MIREN natural vegetation plots in January 2017 and January 2022 through baiting of soils.

Site	Road	Elevation (m)	2017	2022
AK2	Kosciuszko	1050	*P. pseudocryptogea*	
AK4	Kosciuszko	1170		*P. cf. cryptogea*
AK8	Kosciuszko	1405		*P. megasperma*
AS6	Snowy Mts Hwy	680	*P. elongata, P. fallax*	
AS8	Snowy Mts Hwy	790	*P. elongata*	
AS9	Snowy Mts Hwy	860	*P. fallax*	
AS10	Snowy Mts Hwy	900	*P. gondwanense*	
AS12	Snowy Mts Hwy	1030		*P. cinnamomi*
AS17	Snowy Mts Hwy	1320	*P. cactorum*	
AT2	Tooma	500	*P. elongata*	
AT3	Tooma	565	*P. elongata*	
AT4	Tooma	640	*P. elongata*	
AT8	Tooma	860	*P. elongata*	
AT9	Tooma	920		*P. elongata*
AT10	Tooma	980	*P. elongata*	
AT12	Tooma	1100	*P. chlamydospora*	
AT13	Tooma	1140	*P. chlamydospora*	

Discussion

The *Phytophthora* species detected in KNP that are most destructive in native vegetation in Australia are *Phytophthora cinnamomi, Phytophthora gregata* and *Phytophthora multivora*. *Phytophthora cinnamomi* has a very large host range (Cahill et al. 2008) and infection of native plants by this pathogen is a key threatening process under the Commonwealth *Environment Protection and Biodiversity Conservation Act* 1999 and NSW *Biodiversity Conservation Act 2016*; over 100 plant species and about 30 plant communities are regarded as directly threatened by it. *Phytophthora multivora* has been implicated in the death of the dominant forest tree species *Eucalyptus gomphocephala* in Western Australia (Scott et al. 2012), *Wollemia nobilis* in New South Wales (Puno et al. 2015) and *Araucaria* species in Queensland (Shuey et al. 2019). *Phytophthora gregata* is an emerging threat and appears to be prevalent in wetlands and riparian areas, especially in cool climates. It has been commonly detected in the stem collars of dying *Pimelea bracteata* in KNP (McDougall et al. 2018) and is suspected of also infecting and causing death to *Pimelea pauciflora* there. The rapid decline in the *Pimelea bracteata* population warranted its listing as critically endangered on the *Biodiversity Conservation Act 2016*.

Although 15 *Phytophthora* species have now been recorded from baiting in KNP and all are likely to be pathogenic, the current hosts of only two species are known. *Phytophthora cambivora* has been isolated from the roots of symptomatic plants of the alpine shrub *Nematolepis ovatifolia* but unusual climatic conditions are thought to be the primary cause of poor plant health (Green 2016). *Phytophthora gregata* can be very destructive in populations of *Pimelea bracteata* in KNP; however, this shrub has re-emerged since the 2019 / 20 fires despite the continued presence of the pathogen, suggesting that it may be less susceptible when young. The true impact of *Phytophthora* in KNP may be much greater than currently recognised because there has been almost no testing for *Phytophthora* presence in the roots of plants displaying poor health symptoms (and this can be very difficult for large trees).

Some of the *Phytophthora* species detected in KNP are likely to be native to Australia. Indeed, one species, *Phytophthora oreophila* has only been recorded in KNP and may be endemic to the park. Of the other 14 *Phytophthora* species detected from soil baiting in KNP, Burgess et al. (2016) list six as being native to Australia and one species (*Phytophthora cryptogea*) as being of uncertain status. One of the putative native species found in KNP, *Phytophthora gregata*, has more recently been found in several other countries, suggesting it is either introduced to Australia or cosmopolitan. Regardless of origin, some *Phytophthora* species are likely to require management in KNP.

The vegetation at the sites where *Phytophthora cinnamomi* and *Phytophthora multivora* were detected in soil appeared asymptomatic at the time of collection. While these pathogens have a very large host range and are associated with poor vegetation health in many parts of Australia, they may also persist in the vegetation without causing extensive deaths. For instance, common plant species such as *Themeda triandra* and *Rytidosperma* species can become infected by *Phytophthora cinnamomi* but they rarely show symptoms (e.g. Phillips and Weste 1984). This may, however, not always be the case, especially as temperatures rise and more of the park becomes suitable to *Phytophthora* species. Rigg et al. (2018), for instance, found in glasshouse experiments that some subalpine species of KNP (*Epacris petrophila, Grevillea victoriae* subsp. *nivalis, Nematolepis ovatifolium, Oxylobium*

ellipticum, Phebalium squamulosum, Podocarpus lawrencei and *Prostanthera cuneata*) are susceptible to *Phytophthora cinnamomi* infection. In addition, there is evidence that *Phytophthora cinnamomi* can adapt to lower temperatures than are regarded as being ideal for its growth and reproduction; in the laboratory it can be trained to produce zoospores and sporangia at 7.5°C (Khaliq 2019) – i.e., lower than the 9°C minimum temperature for growth documented for this species (Abad et al. 2023). *Phytophthora cinnamomi* will probably coexist with highly susceptible plant species in the coming decades and population declines in some species can be expected.

Phytophthora cinnamomi cannot be eradicated once introduced because it persists in asymptomatic plants after highly susceptible hosts are killed, and there are few options for control (Cahill et al. 2008). The same is likely to be true for other *Phytophthora* species. Given the amount of disturbance in KNP from visitor use (vehicles, walkers, mountain bikes, horse trail rides etc.), construction associated with the Hydro Scheme and ski industry, and widespread large introduced herbivores, preventing further dispersal is likely to be impossible. Despite that, it is worth attempting good hygiene, at least for management and construction vehicles accessing remote areas. Identifying which species need protection and which can be protected using hygiene and quarantine are the first steps in minimising the detrimental impact of *Phytophthora* species in KNP.

The low number of sites where *Phytophthora* species were detected in 2022 precludes any assessment of the effect of fire on these pathogens. Fire can have both positive and negative effects on *Phytophthora cinnamomi* (see McDougall and Liew 2020) but even the hottest fire is unlikely to remove it from soils. The greatest risk with fire is the increase in disturbance and soil movement typically occurring through the construction of firebreaks.

The changes in *Phytophthora* species composition at the plot level we detected between 2017 and 2022 are substantial and highlight the difficulty of monitoring these soil pathogens. However, sampling roots and soils for *Phytophthora* presence at sites with poor plant health is essential for tracking the movement of these and other pathogens in the park. The results of surveys since 2014 provide good baseline data for future assessments of *Phytophthora* distribution.

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